INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

2022 IOCV XXII Abstracts

Abstracts presented at the 22nd Virtual Conference of the International Organization of Citrus Virologists (IOCV), September 7, 14, 21 and 28, 2022 are now available on line at the **Journal of Citrus Pathology (JCP)** (http://journalofcitruspathology.com/).

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22nd Conference of the International Organization of Citrus Virologists, Online, 2022

Abstracts of Presentations

Abstracts presented at the 22nd Conference of the International Organization of Citrus Virologists, presented online in four sessions on 7, 14, 21, and 28 September 2022. The abstracts are arranged in topic sections, in the order in which they were presented. Recommended format for citing abstracts, using the first abstract below as an example, is as follows: da Graça JV. 2022. The centenary of citrus greening in South Africa [abstract]. Journal of Citrus Pathology, 9(2):1. https://doi.org/10.5070/C4.46540

SESSION 1: HUANGLONGBING

KEYNOTE

The centenary of citrus greening in South Africa

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In the late 1920s, citrus growers in two northern regions of South Africa observed new symptoms in the leaves and fruit, thought initially to be nutritional (da Graça et al. 2022; Van der Merwe and Andersen 1937). The rapid spread in the following years indicated a transmissible pathogen (Oberholzer et al. 1965). This was identified in 1965 as vectored by the citrus triozid, *Trioza erytreae,* by which time significant losses were occurring (La Fleche and Bove 1970). Movement of citrus stock to unaffected areas was banned. In 1970, electron microscopy revealed the presence of a microbe in infected phloem tissue (La Fleche and Bove 1970), later identified as an unculturable bacterium *Ca*. Liberibacter africanus (CLaf) (Jagoueix et al. 1994). Attempted control using antibiotics was not successful, and the disease has been managed through the use of certified pathogen-free budwood obtained from the screened Foundation collection in a non-infected area, propagation in screened nurseries, ban on movement of trees into non-infected areas, synchronized area-wide orchard practices (fertilizer, irrigation, pruning) which induce flush, for coordinated vector control, and annual pruning of infected branches and treating to prevent regrowth (Buitendag and von Broembsen 1993). The origin of the disease is likely native rutaceous plants, and several have been discovered to harbor liberibacters which are sub-species of CLaf, but CLaf itself has not been found in any. However, the discovery of one (CLaf subsp. clausenae) in infected citrus in Uganda suggests that this may be an ancestor of CLaf (Roberts et al. 2015). Current concerns are the presence and spread of the Asian form of the disease associated with *Ca*. L. asiaticus and its psyllid vector, *Diaphorina citri*, in east Africa which could impact citrus production in the hotter areas which are less suitable for the temperature-sensitive African form and vector. International coordinated monitoring is occurring in several countries to monitor the psyllid and take appropriate measures.

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The current status of citrus huanglongbing control and research in China

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Due to the rapid expansion of the industry scale and the gradual northward movement of the vector Asian citrus psyllids (ACPs), the Chinese citrus industry is facing a severe challenge of Huanglongbing (HLB). Within the last decade, there was a net increase of 83 counties newly occurred with HLB and the occurred area had increased dramatically during the period of 2014 to 2017 with a summit in 2015. In China, the strategy is to emphasize state-level cooperation for R&D, putting solutions on the first priority. Some outputs for a state key R&D project are listed, which has achieved the desired goal, e.g. in demonstration area of ca 110,000 ha, the HLB incidence decreased to 1.5%, the total amount of pesticides used decreased by 37.5%. Through much effort made, the current HLB status is under control in China. Two typical cases with experienced key points for epidemic areas and two intercepted zones addressed to protect another two superior citrus belts within the recent four years are introduced. Four types of 'Three basic measures +' IPMs are summarized based on categorized areas after a series of demonstrations with integrated prevention and control. Towards basic research, bio-information has been accumulated fast both for *Candidatus* Liberibacter asiaticus (*C*Las) and ACP, some transgenetic materials authorized for field evaluation trials, a few cases on biology of *C*Las effectors, comparison on *C*Las / ACP genomes, and two tech. systems for *C*Las enrichment, have been shown. Finally, three suggestions are made for consideration.

Non-technical summary

Citrus is the largest fruit industry in China, which has faced a serious challenge of Huanglongbing (HLB) within this decade. Although the occurred area with HLB increased, through many efforts made, the current HLB status is under control in China. The key measures experienced, R&D progress and three suggestions have been introduced.

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The threat of huanglongbing to European citrus and means to control disease associated bacteria and vectors

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Huanglongbing (HLB) disease, native to Southeast Asia, has spread in just over a decade to almost every citrus-growing region of the world. China, USA and Brazil, the world's largest citrus-producing countries, are affected by HLB and suffer important economic losses. HLB is caused by three species of phloem-restricted bacteria of the genus *Candidatus* Liberibacter, transmitted by the insect psyllid vectors *Trioza erytreae* and *Diaphorina citri*. *T. erytreae* has been detected in Madeira and in the Canary Islands and since 2014 in the Iberian Peninsula. Currently, *T. erytreae* has spread over the whole Atlantic coast of Spain and Portugal. Moreover, *D. citri* has been detected in Israel. Although the bacteria causing HLB have not yet been detected in the EU, the risk of entry is high, due to the enormous circulation of goods and people, as well as the illegal imports of plant material from citrus and citrus relatives. HLB is considered the most devastating of citrus diseases, due to its rapid dispersion, aggressiveness, economic losses, the difficulty of preventing new infections and the absence of lasting control mechanisms. HLB affects all citrus varieties and rootstocks. Experience indicates that once the vector is present, as soon as the causative bacterium enters, the spread of the disease is unstoppable. It is therefore urgent to take the necessary measures to prevent the introduction of HLB in Europe and, if it does occur, to detect and eradicate it quickly. Pre-HLB is a project funded by the Horizon2020 Framework Programme whose first objective is to prevent the entry of HLB into Europe and to prepare the necessary mechanisms for its effective control in the short, medium and long term. Pre-HLB will develop and implement a holistic contingency plan and will also co-create new solutions to contain the disease through a multidisciplinary approach. Project main goals include improving current HLB surveillance, mitigation and avoidance actions. A global net communication system reaching a wide audience, from farmers to scientists, to prevent HLB-associated organisms introduction/spread has been established. Different eco-friendly control measures have been proven to be efficient in the EU to control *T. erytreae* and the establishment of Citrus Health Management Areas (CHMAs) tools for Spain are close to completion. Research on *T. erytreae* bioecology and HLB-control measures (including discovery of proper resistance/susceptibility gene sources) is ongoing.

Citrus greening management strategies: an APHIS perspective

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Citrus plants are susceptible to a wide range of pests and pathogens. The citrus pests/diseases found in certain areas of the United States include the Asian citrus psyllid (ACP), Huanglongbing (HLB; citrus greening), citrus canker, sweet orange scab, and citrus black spot. APHIS-Plant Protection and Quarantine's Citrus Health Response Program plays a key role in sustaining the US citrus industry. The program safeguards against a variety of citrus pests and diseases in collaboration with growers, states, and researchers, provides guidelines for compliance of citrus nursery stock, fruit inspections, treatments, and certifications, and identifies minimum standards for implementing appropriate surveys, diagnostics, and mitigation measures to reduce the proliferation and spread of diseases of regulatory importance.

Citrus greening is one of the most devastating diseases of citrus. Florida is the most impacted State in the United States. Since 2005, the disease has spread throughout Florida, killing thousands of trees, resulting in a reduction in citrus production of more than 74%. The disease is found in several southern US states. HLB has not yet been detected in Arizona or in commercial citrus in California. Extensive surveys for HLB and ACP, tree removal, treatments, biocontrol releases, restricted movement of citrus fruit and nursery stock are the measures being used to control the vector and prevent further spread of the disease. More recently, the use of antibiotics such as oxytetracycline has shown promise to “cure” trees and improve their productivity, as has soil/root health management. Research is ongoing to identify antimicrobials and mechanisms to deliver these into plants. Gene editing/CRiSPR, and propagation of tolerant trees are also being pursued. Diagnostic protocols are being improved as well as being developed for early detection of HLB, such as detection of the pathogen in the roots. Furthermore, APHIS's International Services conducts survey and biocontrol activities across the border in Mexico for further safeguarding.

In addition to these safeguarding activities, in 2013, the Secretary of Agriculture established the Huanglongbing Multi Agency Coordination group, a unified emergency response framework. The group was established to better position the Department to respond to the citrus industry's immediate and long-term needs in dealing with the disease and to coordinate and prioritize federally-funded research to bridge the gap between research and implementation, reduce unnecessary duplication of research, and more quickly provide practical tools for citrus growers to use. Since 2014 Congress has appropriated funds to support HLB MAC goals to speed the development of tools to help the citrus industry fight back against HLB. The focus has been for shovel-read strategies. Since 2019, APHIS has funded Citrus Research and Field Trials (CRaFT) in Florida, Texas, and California. The purpose of these projects is to determine the best management practices and methodologies to produce citrus under the threat and pressure of HLB and are grower-collaborative multi-year projects. The CRaFT strategies include, field testing various management practices on a large scale, evaluate grove floor management strategies, test psyllid management approaches, and assess the performance of different HLB tolerant and resistant rootstocks and scions. Data collection for these large trials is being provided by USDA's Agricultural Research Services.

Non-technical summary

There are several strategies being implemented in the United States to sustain the US citrus industry by safeguarding against a variety of citrus diseases and pests. This talk provides an overview of various strategies being employed in the United States to prevent the spread of HLB to uninfected areas well as those being employed to manage HLB in areas where it is present. APHIS and State Departments of Agriculture conduct surveys, diagnostics, treatments, and tree removal, employ biocontrol and a clean stock program, while activities by APHIS-International Services across the border protects the United States from the entry of pests and pathogens. In addition, through the HLB MAC program, APHIS supports research and field trials to address the domestic citrus industries immediate and long-term needs in dealing with HLB.

Regional climates influence incidence of '*Candidatus* Liberibacter asiaticus' and *Diaphorina citri* in Brazil

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'*Candidatus* Liberibacter asiaticus' (CLas) (α-Proteobacteria) is phloem-limited pathogen associated with the severe Asiatic form of the destructive citrus disease known as huanglongbing. CLas is transmitted by the Asiatic citrus psyllid, *Diaphorina citri* (ACP). ACP was unintentionally introduced to Brazil before 1936 (Costa Lima 1936). Until CLas was detected in 2004, in the centre of the main citrus belt of Brazil, a continuous area of over 460 thousand hectares within the states São Paulo (SP) and Minas Gerais (MG), it had only been recorded in Asia. In addition to CLas, a previously unknown species of Liberibacter (*Ca*. L. americanus, CLam), also transmitted by ACP, also was detected in 2004. Initially, the incidence of CLam was higher than CLas but within four years CLam incidence fell rapidly to less than one percent. Unlike CLas, CLam is heat sensitive and reaches lower titres in citrus (Lopes et al. 2009)). It is also less efficiently transmitted by ACP. In attempts to suppress HLB, since 2004 symptomatic trees have been eliminated and the orchards frequently sprayed with insecticides to reduce populations of ACP. Despite these efforts HLB continued to spread. Now it is present throughout SP/MG and neighbouring states, and in the bordering countries of Argentina, Uruguay, and Paraguay. In SP/MG, the incidence of symptomatic trees reached 38% in a 2023 survey, an increase of 56% compared to 2022. Failure to reduce the increasing high populations of ACP that have been observed in recent years, caused mostly by ACP resistance to the frequently used pyrethroids and neonicotinoids insecticides, and to eliminate diseased trees leading to an increase in the sources of inoculum, appear to be the main reasons for the increasing incidence of HLB. Nevertheless, in 2023, HLB continued to be irregularly distributed in the major citrus belt. The incidence was considerably higher in the centre-east (54 to 74%) than in the south (11%) or north (less than 0.5% to 1.8%). The northern region is hotter and drier than the other regions, with the rain concentrated during the spring and summer; the south is colder, but the rainfall is more evenly distributed over the seasons; and the centre is in an intermediate situation. Studies indicate that the discrepancy in HLB incidence is caused, at least in part, by this regional climate variation, with impacts on the tree, ACP and CLas. The drier and hotter north limits the frequency and intensity of flushing on the tree canopy and, thus, ACP feeding and reproduction, and the vulnerability of the healthy trees to CLas infection (Cifuentes-Arenas et al. 2018; Lopes et al. 2017). The higher temperatures also limit CLas multiplication in the new flushes and, consequently, the chances of its acquisition by the already reduced numbers of ACP (Lopes et al. 2017; Lopes and Cifuentes-Arenas 2021). In the wetter south there are no strong limitations for the trees to flush or CLas to multiply, but the lower temperatures impact negatively the biology of ACP (Liu and Tsai 2000) resulting in relatively lower numbers of insects. As indicated, the intermediate climate of the centre favours the three agents leading to high incidence of HLB. To better manage HLB, several measures have been implemented. New insecticides against ACP have been used, always in rotation with the traditional ones and, in the regions of lower incidence, symptomatic trees continue to be promptly eliminated. In addition, to reduce risks, growers are moving to states to the west and north of SP and MG and establishing new orchards in areas of very low incidence of HLB, taking with them knowledge gained from two-decades of experience of living with HLB and ACP in the major belt.

Non-technical summary

In the major citrus belt of Brazil, HLB has been irregularly distributed since it was first detected in 2004. Regional variations in rainfall and air temperature are related to incidence of the disease. In the hotter and drier regions, where the HLB incidence is very low, the trees do not produce new shoots as frequently as in the colder and wetter regions, thus reducing incidence of immature shoots and leaves most favourable for ACP to feed and reproduce. Hot environments also limit pathogen multiplication and dissemination. These observations have been useful for growers to improve HLB management and make decisions on where to start new plantings.

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SESSION 2: CITRUS VIRAL DISEASES

KEYNOTE

Three reasons why citrus leprosis is becoming increasingly relevant in Brazil

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Citrus leprosis, caused mainly by the cilevirus citrus leprosis virus C (CiLV-C) and transmitted by tenuipalpid mites *Brevipalpus yothersi*, has occurred in Brazil since the early 1930s. Losses associated with the disease in sweet orange production have been reported for several decades. However, these losses have intensified in recent years, mainly due to aspects related to three main components: 1) the mite vector, 2) the causal agent, and 3) HLB. HLB is considered the main citrus disease worldwide and is currently the number one concern of Brazilian citrus growers. The prioritization of HLB to the detriment of leprosis (and other diseases) impacts its management, leading to a decrease in mite monitoring, an increase in the time it takes to spray acaricides once the action level has been reached, and the use of pesticide mixtures, often reducing the efficiency of pest control (Della Vechia et al. 2022). As a consequence, there has been an increase in mite population densities, which are also favored by fewer molecules available to producers, resistance detected in some populations, inadequate spraying, and warmer and drier weather conditions observed in recent years in citrus production areas due to climate change. In addition, our group has detected significant variability in *B. yothersi* populations, both at the molecular level and in their biology, especially with regard to their vector capacity. Working with individual mites from five isofemale lines, transmission efficiency rates within subpopulations ranged from ~30 to 80% for a given CiLV-C isolate. Finally, the third component that may be playing a role in the increase of leprosis in citrus groves in Brazil is the variability of the virus. Our group has reported and characterized three strains of CiLV-C in citrus orchards that share ~85% nucleotide identities with each other (Chabi-Jesus et al. 2021). One of them, CiLV-C_ASU, was found in a single herborized citrus sample collected in 1937 in Asunción, Paraguay, and kept in the Herbarium of the Biological Institute in Sao Paulo. A survey is underway to determine whether viruses of this strain still occur in nature. The other two strains, CRD and SJP, are easily found in surveys. However, while viruses belonging to the CRD strain are found endemically in citrus orchards throughout Brazil and other American countries, those of the SJP have never been detected outside the citrus belt of São Paulo and Minas Gerais States in Brazil, where their incidence has increased significantly since their characterization in 2016 (Ramos-González et al. 2016). Interestingly, our data show that viruses of the SJP strain are detected up to 20 days earlier in sweet orange seedlings and viral loads consistently reach 10 times those of the CRD. Finally, *B. yothersi* populations transmit SJP up to 64% more efficiently than CRD. Altogether, the growing importance of leprosis is probably the sum of several aspects, mainly associated with the priority given by Brazilian producers to HLB, the increase in the mite´s population density in the field, and the presence of a new and better-adapted strain of CiLV-C in the Brazilian citrus belt.

Non-technical summary

Although citrus leprosis has been considered an important disease for the Brazilian citrus industry for almost a century, in the last few years its relevance has increased due to (i) the prioritization of the growers to control HLB, often to the detriment of proper management of other diseases, (ii) the increase of the vector population density as a consequence of suboptimal management and favored by conducive environmental conditions, and (iii) the presence of a new strain of the causal agent of leprosis, more adapted to the host and the vector.

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The CRP protein encoded by mandiriviruses is a pathogenicity determinant

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The subgenus *Mandarivirus* (genus *Potexvirus*, family *Alphaflexiviridae*) currently includes three members, *Citrus yellow vein clearing virus* (CYVCV), *Citrus yellow mottle associated virus* (CiYMaV), and *Indian citrus ringspot virus*. Compared to other potexviruses, mandariviruses encode an additional cysteine rich protein (CRP). CRP encoded by many plant RNA viruses was demonstrated to be an important pathogenicity factor and suppressor of RNA silencing. However, the function of mandariviruses CRP remains poorly understood. In this study, heterologous expression of CYVCV and CiYMaV CRP with potato virus X (PVX) vector in *Nicotiana benthamiana* resulted in more severe disease symptoms and higher virus accumulation levels than the empty PVX vector. Furthermore, we constructed infectious clones of CYVCV and CiYMaV that successfully infect citrus plants through vacuum infiltration. The inoculated plants developed severe vein yellowing, vein clearing, leaf mottling or dwarfing symptoms by 40 days post-infiltration (dpi). Mutational analysis showed that CRP is required for mandarivirus systematic infection and accumulation in citrus plants and the characteristic motifs (ZF and NLS) of CRP are essential for functioning.

Non-technical summary

Our results demonstrate that mandariviruses' CRP is a pathogenicity determinant affecting viral infection and symptom development.

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24k protection from citrus psorosis virus (CPsV) interferes with plant miRNA biogenesis

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Sweet orange (*Citrus sinensis*) is among the most significant fruit crops globally. Viral infections can disrupt cellular functions, leading to substantial economic losses. We demonstrated that citrus psorosis virus (CPsV)-infected orange plants exhibited lower levels of mature microRNA (miRNA) species and higher levels of unprocessed miRNA precursors and miRNA targets compared to healthy plants (Reyes et al. 2016; Matrmisolle et al. 2020; Marmisolle et al. 2024). 24K protein, the CPsV suppressor of RNA silencing (VSR), interacts with miRNA precursors *in vivo* as revealed by RNA-immunoprecipitation assays (Marmisolle et al. 2018). Thus, this protein becomes a candidate responsible for the increased accumulation of unprocessed miRNAs. We also showed that 24K colocalizes within nuclear D-bodies with the miRNA biogenesis proteins DICER-LIKE 1 (DCL1), HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE). According to results of BiFC and CoIP assays, the 24K protein interacts with HYL1 and SE (Marmisolle et al. 2024). Thus, 24K may inhibit miRNA processing in CPsV-infected citrus plants by direct interaction with the miRNA processing complex. This study enhances the understanding of how a virus can disrupt the host's regulatory processes, specifically targeting miRNA biogenesis and functionality.

Non-technical summary

Sweet orange may suffer from disease symptoms induced by virus infections, thus resulting in drastic economic losses. Infection of sweet orange plants with CPsV, alters the accumulation of a set of precursors from the important regulatory molecules called miRNAs. This processing misregulation is explained by direct association of one of the viral proteins (24K) with miRNA precursors and also by its interaction with components of the cell miRNA processing machinery leading also to alteration in miRNA target transcripts.

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Advances in controlling citrus yellow vein clearing disease

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Citrus yellow vein clearing virus (CYVCV) is an emerging virus that causes vein clearing, leaf crinkling, occasional veinal necrosis, and abscission of leaves in lemon (*Citrus limon*), sour orange (*C. aurantium*) and some cultivars of satsuma (*C. unshiu*). The symptoms induced by CYVCV can be reduced at high temperatures (Zhou et al. 2016). Since it was first recognized in Yunnan Province of China in 2009 (Chen et al. 2014), CYVCV has been reported in all major citrus-growing provinces of China because it is easily transmitted by grafting, contaminated tools, *Dialeurodes citri*, and several aphid species (Afloukou et al. 2021; Zhang et al. 2018; Zhang et al. 2019 a and b). Currently, CYVCV is considered the most important virus affecting lemon production in China (Wang et al., 2022). In addition to members of the citrus genus, CYVCV has been reported to infect herbaceous plants, such as *Phaseolus vulgaris* and *Vigna unguiculata* where it can cause chlorosis, severe mosaicism of leaves, and general necrosis (Önelge et al. 2011). CYVCV has been detected

on weed species including *Malva sylvestris*, *Solanum nigrum*, *Sinapis arvensis*, and *Ranunculus arvensis*, but infected plants are largely symptomless (Önelge et al. 2016).
The CYVCV genome consists of a highly conserved positive-sense RNA of approximately 7.5 kb, with six predicted open reading frames (ORFs) (Loconsole et al. 2012). ORF1 encodes a putative RNA-dependent RNA polymerase (RdRp). Triple gene block proteins (TGBp1, TGBp2, and TGBp3), encoded by ORFs2-4, are involved in viral movement (Loconsole et al. 2012). The coat protein (CP) encoded by ORF5 has been identified as an RNA silencing suppressor (RSS) and is associated with the pathogenicity of CYVCV (Bin et al., 2022; Rehman et al., 2019). Viral CP colocalizes with TGBp1 and TGBp3 at the plasmodesmata (PD) of the epidermal cells of *Nicotiana benthamiana* (Rehman et al. 2019). ORF6 overlaps with ORF5 and encodes a hypothetical 23 kDa protein with an unknown function (Loconsole et al. 2012). Although CP interacts with all TGB proteins (Rehman et al. 2019), few studies have investigated the interaction between CYVCV-encoded proteins and host factors. Zeng et al. (2023) identified the interaction of ClRPS9-2 with CP, and a conserved domain of ClRPS9-2 was identified as indispensable for its interaction with CP. Transient expression of ClRPS9-2 reduced the CP content and its ability as a silencing suppressor in *N. benthamiana*. Furthermore, the resistance to CYVCV in transgenic Eureka lemons was enhanced by over-expressing ClRPS9-2.

Measures to limit damage due to CYVCV include the use of virus-free propagation material, control of vectors (aphid and citrus whitefly), removal of diseased trees and disinfection of tools.

Non-technical summary
CYVCV is an emerging citrus virus and is now considered to be the most serious pathogen affecting lemon production in a few countries such as Turkey, India and China. The recent research findings on CYVCV occurrence, distribution, host range, transmission, gene function, host-virus interactions and control measures were reviewed.

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SESSION 3: CITRUS VIROIDS

KEYNOTE

Transcriptome and microRNA analysis in dwarfed citrus trees infected with citrus dwarfing viroid

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Citrus dwarfing viroid (CDVd) can reduce the canopy volume of navel orange trees on trifoliate rootstock (approx. 50%), increase the yield per canopy volume, and concentrate fruit in the optimum canopy zone for harvest without affecting fruit quality. To observe the long-term effects of CDVd on canopy volume, a survey of navel orange tree growth was conducted in an experimental block planted in 1998 at the Lindcove Research and Extension Center, in central California, and found that the vegetative apical shoot growth of CDVd-treated trees was reduced by almost 20% compared to the non-treated controls. Dwarfed trees are fundamental for high-density plantings, which will be critical to meeting challenges posed by water shortages, disease spread, farmland reduction, and increasing labor costs. To gain insight into the molecular mechanisms modulated by CDVd, we performed microRNA and transcriptome analyses in dwarfed citrus trees infected with CDVd and compared them to the non-infected controls. Three plant miRNAs (csi-miR479, csi-miR171b, and csi-miR156) were significantly downregulated in CDVd-positive stems, and potentially linked to the characteristic dwarfed phenotype. Differential expression analysis identified genes associated with various physiological and developmental processes, including upregulated transcription factors (MYB13 and MADS-box) and downregulated calcium-dependent lipid-binding protein. Transcriptome reprogramming primarily occurred in the scion rather than the rootstock, supporting the role of CDVd as a specific gene-modifying agent rather than a universal disease-causing pathogen. These results provide insights into the genetic mechanisms behind citrus dwarfing, crucial for future citricultural developments for high-density, dwarfed, profitable, and sustainable citrus orchards.

Managing viroids in Australasia

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The responsibility for citrus biosecurity lies largely with the Australian government through border protection, surveillance, and post-entry quarantine for imported citrus plant material of new varieties. Biosecurity is also a priority for the Australian citrus industry, supported by industry-driven preparedness, awareness, and surveillance activities, in addition to funding research to enhance diagnostics and management.

Imported and Australian selections of new varieties are tested and cleared of all citrus viroids and other 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. The repository programme is managed by the Auscitrus propagation scheme, which also maintains pathogen-tested and true-to-type daughter trees for commercial supply of propagation material. Trees are regularly tested for viroids and other graft-transmissible pathogens by the New South Wales Department of Primary Industries (NSW DPI). This initiative between industry and government to manage graft-transmissible pathogens in citrus has been evolving since the establishment of the Cooperative Bud Selection Society in 1928. However, the use of health-tested propagation material is not mandatory in Australia.

The following viroids have been reported in citrus cultivars in Australia: citrus exocortis viroid (CEVd), citrus bent leaf viroid low sequence similarity (CBLVd-LSS), hop stunt viroid (HSVd), citrus dwarfing viroid (CDVd), citrus viroid V (CVd-V), citrus viroid VI (CVd-VI) and citrus viroid VII (CVd-VII). Studies on the newly discovered CVd-VII are building our understanding of its incidence and biosecurity risk. A strain of CDVd (IIIb) is available for commercial use in high density plantings in Australia. Field trials were recently established to determine the long-term impact of the commercial dwarfing viroid with newly detected or discovered viroids under Australian conditions.

The National Citrus Repository house at NSW DPI’s Elizabeth Macarthur Agricultural Institute not only supports the Australian industry, but it has been an offshore plant quarantine facility for New Zealand since 2001, allowing new citrus varieties to be imported into a lower level of quarantine in New Zealand from Australia if found free from specified regulated pests prior to export. Long-term, collaborative project work between Australian and local scientists in Bhutan, Lao People’s Democratic Republic, Thailand, Pakistan, and Indonesia aimed to enhance the understanding and management of graft-transmissible citrus pathogens, including viroids.

Non-technical summary

Viroid diseases are managed in Australia by ensuring industry has access to healthy propagation material to prevent orchard infections. Auscitrus sells budwood from pathogen tested trees, but it is not mandatory to use this material for propagation.

Citrus viroids in commercial settings in South Africa

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The South African citrus industry produces a range of citrus types in a changing landscape, shaped by market demands. Top-working is an industry practice that is used at times to change a scion cultivar of an existing orchard. The advantage of top-working is that a well-established root system can accelerate growth of the newly budded scion. There is no need for land preparation and full production can be achieved earlier compared to newly planted trees. At this stage, it is estimated that 1-2% of total hectares are top-worked. It might be a practice that becomes more widely used in a dynamic market

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environment. Therefore, viroids may start impacting our industry to a greater degree. South African growers are somewhat naïve regarding the impact of viroids as we have a healthy industry due to the functioning of the Citrus Improvement Scheme (CIS). In recent years however, we investigated various scenarios where viroids severely impacted orchards, either due to the use of field-cut material or top-working older, viroid-infected orchards. Severe stunting of new mandarin orchards was associated with citrus dwarfing viroid (CDVd) and hop stunt viroid (HSVd) on both Carrizo citrange and Citrange 35 rootstocks due to the use of infected field-cut bud-wood. Another occurrence was a top-work of an older Valencia orchard to a mandarin scion which was then severely impacted by cachexia. HSVd was latent in the original Valencia scion. These incidences are warnings to the 'hidden dangers' of changing practices.

Our viroid research is focusing on rootstock susceptibilities, since the range of rootstock cultivars has increased in recent years, but pathogen susceptibilities are largely unknown.

The objective of a field-trial was to compare tree health, production and fruit characteristics of CIS supplied material with that of field-cut and viroid infected material. Budwood was collected from original field sources of three cultivars, which contained various populations of CTV strains and citrus viroids. These were budded to Swingle citrumelo, Carrizo citrange and Citrange 35 rootstocks. The same was done for budwood obtained from the CIS and for CIS budwood to which a non-cachexia causing hop stunt viroid source was additionally inoculated. The field trial was established in 2016 and after six and a half years, significant differences in tree growth were observed between treatments. Significantly reduced canopy volumes were associated with field-cut material of all three cultivars, but also with the HSVd treatment. Trees made from CIS supplied budwood were consistently the largest. The 2022 season was the fourth harvest from the trial. The average, four-year cumulative yields of trees made with CIS budwood were correlated to tree size and were significantly higher than yields obtained from trees made with field-cut material, but also compared to the HSVd treatment. The effect of dwarfing and yield reduction of the viroid treatments differed between rootstocks and cultivars. Carrizo, the most used industry rootstock was most affected by viroids.

Non-technical summary

Viroids can negatively impact growth and yield, but outcomes are unpredictable due to variables of different scions, rootstocks and viroid species as well as timing of infection. Top-working tolerant rootstocks is a viable practice, but prior viroid screening of orchard infections is advisable. Viroid-free budwood should be used for tree production.

Citrus viroids in Northwestern Argentina: an overview

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Argentina has two different citrus producing areas, with different climate, soil and citrus varieties. From the total country citrus production, 91 % of lemons, 35 % of grapefruits and 17 % of sweet oranges are produced in the Northwestern region. The Estación Experimental Agroindustrial Obispo Colombres has strongly contributed to the citrus industry regional development and its leading position. The sanitary status in relation to graft transmissible diseases is high due to the use of nucellar clones introduced during the 1960´s and the national citrus certification program, implemented since 2010. Nevertheless, because almost 60% of citrus orchards planted are more than 12 years of age, it is possible to find plants infected with viroids in the field. Since 2003, in Tucumán, Salta and Jujuy provinces, citrus samples were collected from symptomatic field plants, asymptomatic plants in plots with viroid-infected plants, plants infected with other pathogens and plants selected based on information obtained in trials. Samples were tested using biological and molecular methodologies following standard protocols. Initially, biological indexing was done exclusively, and symptoms obtained were variable and ranged from mild to very severe. In 2006, sequential PAGE methodology was incorporated, confirming that most of the infections were mixed, and, in addition to the regulated viroids of citrus exocortis viroid (CEVd) and hop stunt viroid (HSVd), citrus plants were infected with other viroids that had not previously been reported. In 2012, RT-PCR methodology for diagnosis and identification of HSVd and CEVd was performed and in 2018 specific primers for citrus bent leaf viroid (CBLVd), citrus dwarfing viroid (CDVd), citrus bark cracking viroid (CBCVd) and citrus viroid V (CVd-V) were included. To date, 22 lemon, 17 grapefruit, 15 orange, 2 Cleopatra mandarin, 2 citrumelo (Swingle and 75 AB), 1 Tahiti lime and 1 Cape Narge tangerine plants have been tested. Results show that viroids present in the region are CEVd, HSVd, CDVd and CBLVd. They were detected in single and mixed infections but most trees contained at least two viroids. CVd-V and CBCVd were not found in any sample analyzed. CDVd was predominant, present in all varieties and rootstocks and CBLVd was found in all grapefruit plants tested and only in five old lemon trees. This was the first CBLVd local detection in lemon and plants showed atypical symptoms of severe bark scaling in the cultivar. The viroid survey is ongoing.

Non-technical summary

Sixty citrus samples including 22 lemons, 17 grapefruits, 15 oranges, 2 citrumelos, 2 Cleopatra mandarin, 1 tangerine and 1 Tahiti lime from Northwestern Argentina have been collected and tested for viroid presence since 2003. Results show that viroids present are CEVd, HSVd, CDVd and CBLVd, in single and mixed infections. CDVd was predominant, present in all varieties and rootstocks, and CBLVd was found in all grapefruit plants tested and only in five old lemon cultivars. CVd-V and CBCVd were not found.

SESSION 4: OTHER CITRUS DISEASES, INCLUDING EMERGING

KEYNOTE

Snapshot: Phantom agents and emerging pathogens

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Phantom pathogens in citrus are presumed virus or virus-like agents historically believed to cause specific disorders, despite limited studies, scarce literature, undefined etiologies, and the absence of type isolates, which precludes the development of detection assays. These phantom agents are often embedded in regulatory frameworks, impacting international trade and complicating the global movement of citrus germplasm, while also causing confusion within the scientific community and literature. To address these issues, a dedicated team of members of the International Organization of Citrus Virologists (IOCV), conducted a systematic review to assess the validity of these phantom agents by analyzing hundreds of historical reports and consulting global experts. The team identified 55 phantom cases across three categories: disorders with no identified causative agent, diseases of unknown etiology with documented or potential economic importance, where a causal virus or agent was subsequently identified, and older virus-like diseases likely eradicated through advancements such as thermotherapy, shoot-tip grafting, and pathogen-free propagation programs. These include disorders documented between 1913 and 1972, which no longer appear in the literature following the implementation of these techniques and regulatory indexing programs in the 1970s. By consolidating these findings into a white paper, this effort on citrus phantom disorders aims to clarify the scientific literature, guide regulatory agencies in updating pathogen lists, reduce unnecessary restrictions, and align citrus plant health standards with current diagnostic capabilities. This refined, science-based approach ultimately supports the sustainable trade and resilience of the citrus industry while maintaining rigorous plant health protections, with a focus not on phantoms of the past, but on the newly characterized and emerging graft-transmissible pathogens that pose a threat to citrus.

Phytoplasmas and phytoplasma diseases in citrus

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Phytoplasmas are wall-less bacteria which multiply in phloem sieve-tubes and are transmitted by phloem feeding insect vectors and by vegetative propagation, grafting, cuttings and, in some species, also by seeds (Satta et al., 2019). Citrus species are reported to be infected worldwide by phytoplasmas enclosed in 11 ribosomal groups (Noorizadeh et al. 2022). Insect vectors of '*Candidatus* Phytoplasma aurantifolia', the first identified phytoplasma in citrus, are *Hishimonus phycitis* and *Diaphorina citri*. Seed transmission was reported in one case of lime witches' broom (Satta et al. 2019). Recently the presence of '*Ca*. P. palmae' phytoplasma, associated with coconut lethal yellowing, was detected in citrus orchards in the Caribbean areas, mainly in Cuba, and this phytoplasma was also isolated in artificial medium (Luis-Pantoja et al., 2021).

During surveys in the Caribbean from 2017-2020, almost 100% of citrus plants were found to be infected with '*Ca*. Liberibacter asiaticus' ("huanglongbing", HLB). In Cuba, 22% of them were also infected with 16SrI, -IV, -VII, -XI and -XII phytoplasmas, and in Guadeloupe, 13% of the phytoplasma-infected plants had a mixed infection with HLB. Moreover, only phytoplasmas were detected in some samples of symptomatic citrus indicating that the symptomatology cannot discriminate between the two pathogens nor among phytoplasma ribosomal groups. Phytoplasma presence in citrus represents a source of infection for other crops increasing the difficulties in preventing their spread to the surrounding vegetation. Their presence seems to be as dangerous as the presence of HLB agents '*Ca*. L. asiaticus', '*Ca*. L. americanus' and '*Ca*. L. africanus'. The use of healthy propagation materials, together with specific and sensitive testing of symptomatic field trees, will help to reduce the spread of these pathogens and their economic damage.

Non-technical summary

Phytoplasma presence in citrus was associated with epidemics in citrus-growing regions of the world in orange, lime and lemon, grapefruit, and mandarin. They are associated with either specific symptoms such as witches' broom or non-specific symptoms like leaf yellowing, stunting and decline. So far phytoplasmas enclosed in 11 ribosomal groups were detected and among these 16SrI (aster yellows) and 16SrII (peanut witches' broom) are the most widespread. It is not uncommon to find mixed infections of diverse '*Candidatus* Phytoplasma' and '*Candidatus* Liberibacter' species with identical symptomatology that requires specific testing for appropriate disease management.

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The immigrant's story: the arrival of citrus black spot in Florida and what that revealed

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Citrus black spot (CBS), caused by the fungus *Phyllosticta citricarpa*, was first described in southwest Florida in early 2010 (Schubert et al. 2012), where it has largely remained. While looking for airborne ascospore inoculum in Florida, it was discovered that *P. citricarpa* was heterothallic and only one mating type (MAT1-2) was present (Wang et al. 2016), the same sole mating type later described in Cuba (Serra et al. 2022). This meant that the only form of inoculum present was the asexual, splash dispersed conidia, something only once previously reported (Whiteside 1967) but not genetically confirmed. The evidence for the clonal *P. citricarpa* population with only MAT1-2 present was strengthened by two subsequent global population structure studies (Carstens et al. 2017; Coetzee et al. 2022). Tran et al. (2017) used the mating type evidence to demonstrate the role of spermatia as male gametes, produced the first *P. citricarpa* ascospores in culture, and confirmed recombination by genotyping individual ascospores. When following inoculum production in Florida leaf litter, 10% of the leaves were found to contain structures of *P. citricarpa* and the morphologically similar endophyte, *P. capitalensis*. Leaf decomposition stage affected the type of fungal structure found with pycnidia occurring at lower levels whereas pseudothecia were formed at high levels of decomposition. In each season from 2014 to 2017, the number of leaves with pycnidia increased but the number with pseudothecia did not change substantially. This was of interest because *P. citricarpa* and *P. capitalensis* produce pycnidia but only *P. capitalensis* produced pseudothecia indicating *P. citricarpa* was becoming more common in the grove while *P. capitalensis* levels were largely unchanged. This conclusion was confirmed by qPCR identification of both fungi in the leaf samples. While a substantial quantity of *P. citricarpa* conidia are produced in the leaf litter, the source of canopy infection remains unclear. Low levels of *P. citricarpa* conidia were collected from dead twigs over two years. However, the quantity of *P. citricarpa* DNA from the twig bark exhibited a similar pattern to fruit severity ratings on the same trees. Many questions remain about the sources of *P. citricarpa* conidia responsible for CBS epidemics in Florida.

Non-technical summary

Citrus black spot is an important fungal disease in many regions of the world. The Floridian CBS outbreak is caused by only one spore type reported to spread short distances. Large quantities of these spores were found in the leaf litter but far fewer in dead twigs in the canopy. It is not clear whether one or both spore sources are important for spread and disease persistence in Florida.

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Synergistic mechanisms of CsWRKY22 and CsLOB1 plant growth regulators against citrus canker disease

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Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (Xcc), is a severe bacterial disease affecting most of the commercially important citrus cultivars. The canker symptoms in sweet oranges, lemons and limes include pustule formation on the surface of leaves, stems and fruits. Pathological hyperplasia and hypertrophy of host cells are the primary conditions for pustule formation, but their regulators are largely unknown. Here, we reported the roles of CsLOB1 and CsWRKY22 transcription factors in the regulation of citrus development and susceptibility to citrus canker. CsLOB1-overexpressing plants exhibited dwarf phenotypes with smaller and thicker leaves, increased number of branches and adventitious buds clustered on stems. These phenotypes also exhibited enhanced cell proliferation resulting in the development of pustule- and canker-like formations, confirming that CsLOB1 can alone induce canker-like symptoms in healthy plants. CsWRKY22-overexpressing plants also exhibited similarly dwarf phenotypes, but these phenotypes were accompanied by increased cell size in the spongy mesophyll. The data showed that CsLOB1 and CsWRKY22 triggered citrus cell proliferation and enlargement, respectively. Resistance evaluation confirmed that both CsLOB1 and CsWRKY22 positively regulated plant susceptibility to citrus canker. Interestingly, CsWRKY22 upregulated the expression of CsLOB1 through directly binding to the W-boxes just upstream of the transcription start site of CsLOB1. Further, our data indicated that CsLOB1 and CsWRKY22 participated in the regulation of cell wall degradation and remodeling and hormone (such as cytokinin, auxin and brassinosteroid) pathways. In summary, our study revealed that CsWRKY22 and CsLOB1, mainly as plant growth regulators, synergistically promoted Xcc-induced pustule formation by mechanisms based on cell wall degradation and remodeling and phytohormone signaling, which may be critical to citrus canker development and bacterial growth in citrus.

Non-technical summary

Using CsLOB1 as the central regulator, CsWRKY22 and CsLOB1 cascade regulates the hypertrophy and hyperplasia of citrus cells and favors host disease susceptibility.

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Mal secco disease of citrus: will it forever be the mediterranean basin problem only or is it coming to other citrus growing areas of the world?

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The citrus disease known as 'mal secco' is caused by the mitosporic ascomycete fungus *Plenodomus tracheiphilus*, formerly classified as *Phoma tracheiphila* (Petri). This destructive vascular disease primarily affects lemon and other citrus varieties and is currently limited to the Mediterranean basin, causing significant economic impact on the citrus industry. The fungal pathogen enters the host tree through wounds in the roots or canopy. Infection rapidly spreads through the canopy into the main branches and trunk, often leading to tree mortality. Key symptoms include veinal chlorosis, leaf wilt, red coloration of the xylem, and dieback of twigs and branches. The prevailing methods for disease control involve sanitation of infected wood and copper application during winter to prevent germinating spores from infecting the plant. Recently, we have developed a drip irrigation protocol using Futriafol, aiming to protect trees and control the disease.

A study projecting the disease's potential distribution in the Mediterranean basin under current and future climate conditions indicates a 23% overall decrease in suitable habitat for the pathogen by 2070, yet a significant portion of the Mediterranean basin will still remain susceptible to infection.

While mal secco is currently confined to the Mediterranean basin, there is a concern about its potential spread beyond this region. The disease's origin is traced back to Southeast Asia (India-China), where favorable conditions exist, yet no reports of the disease have emerged from this area. With changing climate conditions and easier global movement of plant material, there is a possibility that the pathogen could spread to citrus-growing regions where it is currently absent but conditions for disease establishment are present. A proactive approach is crucial in preventing such scenarios in these areas. In my talk, I describe the disease symptoms and raise the question of its spread to other parts of the globe.

Non-technical summary

Mal secco disease is a devastating disease affecting lemons and citrus overall. At the moment, it is contained within the Mediterranean basin, but there is a real concern it could spread to other countries where citrus is grown. This worry arises from changes in climate that might create suitable conditions for the disease in new parts of the world, and due to movement of plant material between countries that can act in the spread of the pathogen, potentially leading to infections in new orchards. It is crucial to recognize and address these risks to protect citrus crops worldwide.

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