



Citation: B. Giovani, A.-L. Boutigny, K. Djelouah, A. Fox, A.M. D'Onghia (2022) Plant Health research collaboration in the Mediterranean region: case studies on citrus tristeza virus, tomato brown rugose fruit virus and *Xylella fastidiosa*. *Phytopathologia Mediterranea* 61(3): 525-530. doi: 10.36253/phyto-14085

Accepted: December 14, 2022

Published: January 13, 2023

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

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60th MPU Anniversary Special Section - Current topics

Plant Health research collaboration in the Mediterranean region: case studies on citrus tristeza virus, tomato brown rugose fruit virus and *Xylella fastidiosa*

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Summary. Strengthening Plant Health research is a major challenge for Mediterranean countries. The diversity and fragmentation of the research landscape in this region have weakened the impacts of national efforts. Mediterranean countries can benefit from coordination of research activities to increase efficiency and impacts. The initiative 'Plant health research priorities for the Mediterranean region', led by the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM Bari)¹ and the Euphresco network for phytosanitary research coordination and funding² can promote convergence of national programmes and optimize the use of the scarce funding available to plant health, thus strengthening international cooperation and increasing the excellence and relevance of research.

Keywords. Research coordination, international collaboration, science diplomacy.

INTRODUCTION

Approximately 25,000 plant species inhabit the Mediterranean region, and of these 13,000 are endemic. The region has been recognised as a biodiversity hotspot that is suffering unprecedented loss of habitat (Myers *et al.*, 2000).

¹ CIHEAM Bari, International Center for Advanced Mediterranean Agronomic Studies, (<https://www.iamb.it/>), is an intergovernmental organization for high education, applied scientific research and planning of partnership actions in the field of research and international cooperation in the Mediterranean region.

² Euphresco, Network for phytosanitary research coordination and funding (<https://www.euphresco.net/>), is an international network of research programme owners, programme managers, policy makers, regulators and research and educational organizations from ca. 60 countries in 5 continents.

Mediterranean agriculture, forests and other environments are threatened by numerous quarantine and emerging pests. The negative impacts of these organisms have increased due to global trade and climate change that favour their movement over long distances and facilitate their survival in previously unfavourable environments. The EU Farm to Fork strategy, with its target to reduce by 50% the use and risk of chemical and hazardous pesticides by 2030, and the rise of biological crop products market, have also reduced the availability (if any) of control measures for some quarantine or emerging pathogens. In the face of these challenges, the Mediterranean region is particularly vulnerable.

The political and cultural diversity that are a characteristic of the Mediterranean region are its strength and its weakness. Diversity creates fragmentation, is an obstacle to collaboration, and reduces opportunities for concerted actions, which can result in individual countries having to deal alone with problems. The International Centre for Advanced Mediterranean Agronomic Studies of Bari (CIHEAM Bari) and the Euphresco network for phytosanitary research coordination and funding joined forces in 2016 to help Mediterranean countries to rethink organization of research activities and their coordination, to increase the efficiency and impacts of national efforts.

The benefits of research coordination and international collaboration are diverse. Identifying research priorities (important pests, infrastructure and capabilities) at the supra-national level will enhance convergence of national and regional programmes and will allow research funders to take advantage of the optimization of the scarce funds dedicated to plant health. Regulators will benefit from reinforced links with research funders and researchers, and from research support for policy development. Scientists will benefit from international knowledge exchange projects, from opportunities for enhancing their science capability and from increased relevance and visibility of plant health (research) activities.

THE CIHEAM BARI AND EUPHRESCO INITIATIVE

The approach followed by the CIHEAM Bari and Euphresco to identify the plant health research priorities for the Mediterranean region merged research experience and national guidance. A survey collected opinions of national experts from Mediterranean countries. The survey resulted in lists of pests, research priorities, infrastructures and research capacity that are considered important for the region. The priorities from the survey were refined and complemented by taking into account the short- and medium-term (up to 5 years) national research pro-

Table 1. Priority pests for the Mediterranean region, identified through a survey.

Selected pests (in alphabetical order)
<i>Anoplophora chinensis</i>
<i>Bursaphelenchus xylophilus</i>
' <i>Candidatus</i> Liberibacter africanus'
' <i>Candidatus</i> Liberibacter americanus'
' <i>Candidatus</i> Liberibacter asiaticus'
' <i>Candidatus</i> Liberibacter solanacearum'
Citrus tristeza virus
<i>Drosophila suzukii</i>
<i>Erwinia amylovora</i>
<i>Fusarium oxysporum</i> f.sp. <i>albedinis</i>
<i>Phyllosticta citricarpa</i>
Plum pox virus
<i>Rhynchophorus ferrugineus</i>
<i>Spodoptera frugiperda</i>
Tomato brown rugose fruit virus
<i>Xylella fastidiosa</i>

grammes, which provided information on the more urgent research topics planned for funding in each country. The views of national regulators provided additional guidance for selecting the most relevant priorities (Table 1).

Workshops and consultations were organised to involve and seek the endorsement of high-level representatives from Mediterranean countries, international organizations, and initiatives, that represent research funders, policy makers and research organizations in the Mediterranean region, including the Arab Society for Plant Protection (ASPP)³ and the Mediterranean Phytopathological Union (MPU)⁴ (D'Onghia *et al.*, 2022; Giovanni *et al.*, 2022). Given the large number of priorities and the limited human and financial resources available, it was agreed that the research effort would focus on a small number of research topics. These shortlisted topics were those that received the largest number of expressions of interest from organizations in the four Mediterranean regions of Balkan-Mediterranean, Eastern Mediterranean, Maghreb, and Western Mediterranean. The three projects are outlined below.

³ ASPP, Arab Society for Plant Protection (<https://www.arabspp.org/>), is an organization of scientists from public and private academic institutions and from industry that promotes research, education, and extension activities related to pests in Arab speaking countries

⁴ MPU, Mediterranean Phytopathological Union (<http://www.mpunion.eu/>), is a regional not-for-profit organization that aims to advance and disseminate knowledge on phytopathology and closely related fields relevant to Mediterranean agro-ecological regions, and to establish and foster research relationships among scientific societies, academia, scientists and stakeholders

Rapid and efficient detection and identification of citrus tristeza virus (CTV) isolates that induce severe symptoms on Citrus

This research initiative aims to simplify the diagnosis of CTV isolates that cause severe symptoms on *Citrus*. To date, six major CTV phylogenetic groups have been described, including: T36 (Karasev *et al.*, 1995), T3 (Hilf and Garnsey, 2002), VT (Mawassi *et al.*, 1996), T68 (Harper, 2013), T30 (Albiach-Marti *et al.*, 2000), and RB (Harper *et al.*, 2010). These groups are mainly based on their genomic features. Virus genotype variation may occur after the passage through different hosts and CTV genotype populations may influence the success of virus transmission by the vector *Aphis gossypii* (Camps *et al.*, 2022). Some of CTV genotypes are known to cause severe symptoms in *Citrus* orchards and have restricted distribution in the EPPO region. As molecular tests alone are of limited value for the prediction of pathogenic properties of CTV isolates (Bar-Joseph *et al.*, 2010; Harper, 2010), the diagnosis of CTV isolates that induce severe symptoms on *Citrus* has been classically performed using a combination of molecular, serological and/or biological tests. This protocol is lengthy and difficult to perform. Moreover, the monoclonal antibodies MCA13 (Permar *et al.*, 1990) used to diagnose CTV isolates that cause severe symptoms are no longer commercially available, which negatively impacts the diagnostic protocol currently in use. During this project, information on the diagnostic molecular tests available or in development will be collected, and plant material from the field (infected, healthy, symptomatic, asymptomatic) will be sampled, following a common methodology for monitoring. In addition, relevant plant material from reference CTV collections will also be collected. The CTV isolates will be molecularly characterized, and sequence data will be used to design the primers and the probes. A test performance study will be organized to validate the tests available or in development, and the test developed in the framework of the project. Several countries have expressed interest in participating in the project, including Australia, Austria, Croatia, Egypt, France, Greece, Israel, Italy, Morocco, Palestine, Portugal, Spain, Switzerland, and Tunisia.

Insights into the biology of tomato brown rugose fruit virus: virus survival in soil

Tomato brown rugose fruit virus has rapidly emerged from initial outbreaks in Jordan and Israel (Salem *et al.*, 2016; Luria *et al.*, 2017), and has now been reported from multiple countries across the Northern

Hemisphere (EPPO, 2022). The virus particles are stable and can survive for months outside hosts on inert and biological surfaces as well as in nutrient film solutions and soil, without losing their virulence (Skelton *et al.*, 2021). Studies on the survival of this virus have been ongoing in Israel (Dombrovsky *et al.*, 2022), but additional information is required, including effects of soil type, environmental conditions, crops cultivated and the management practices. The project aims to develop new knowledge on survival of the virus in soil in different agro-ecological and pedoclimatic conditions. Survival during composting for bioremediation will also be considered within the project. Tests will be validated for the diagnosis of the Tobamovirus in soil and compost (including eDNA approaches), considering approaches to confirm infectivity of detected viral nucleic acids. These data will support development of guidelines for management of the virus. Several countries have expressed interest to participate in the project, including Australia, Austria, Chile, Germany, United Kingdom, Ireland, Israel, Italy, the Netherlands, New Zealand, Palestine, Russia, Slovenia, Switzerland, and Turkey.

Diagnosis of Xylella fastidiosa: detection on dormant plant species which are important for Mediterranean countries

Several research projects on *Xylella fastidiosa* have been commissioned since 2015, by national and regional funders in Europe. This has allowed development of knowledge on the bacterium, which has been useful for developing guidelines for sampling and diagnostics. Hopkins (1981) concluded that sampling should be performed during the period of active plant growth to maximize the likelihood of detection. However, recent experiments have shown that in Mediterranean countries, *X. fastidiosa* can be detected in plants (such as olive, almond and cherry) throughout the year, and especially during the asymptomatic phases or host dormancy, the period with the lowest bacterial concentrations (D'Onghia *et al.*, 2022). The Euphresco project aims to evaluate the distribution dynamics of *X. fastidiosa* within dormant Mediterranean plant species and matrices that are commercially important throughout the year and during dormancy on woody host stems. During the project, samples from dormant Mediterranean plant species that are hosts of *X. fastidiosa* will be collected. An inventory will be made of tests (sampling, DNA extraction, diagnostic tests) used by different laboratories to detect *X. fastidiosa* in dormant plants. Selected tests will be validated on spiked and naturally infected host samples at low bacterial concentrations. Distribution dynamics of the pathogen within natu-

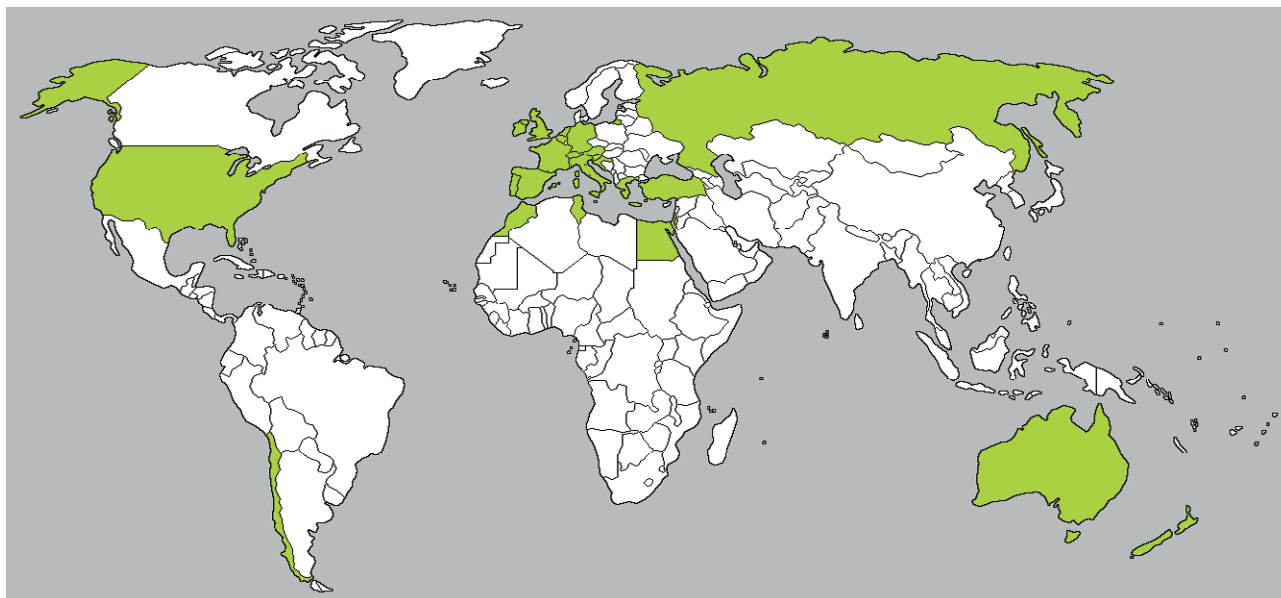


Figure 1. Countries that have expressed interest to participate in at least one of the research projects commissioned through Euphresco on research priorities for the Mediterranean region.

rally infected woody stems will be assessed throughout the year (including during dormancy), if plant material is available. Several countries have expressed interest to participate in the project, including Australia, Belgium, Egypt, France, United Kingdom, Israel, Ireland, Italy, Morocco, the Netherlands, Slovenia, Spain, Switzerland and Turkey.

GEOGRAPHIC EXTENT OF THE PROJECTS

The geographic coverage of the three research consortia is presented in Figure 1.

CONCLUSIONS

The Strategic Framework 2020–2030 of the International Plant Protection Convention (IPPC)⁵, adopted at the 15th Session of the Commission on Phytosanitary Measures (CPM-15) in April 2021, includes global phytosanitary research coordination as one of the eight development agenda items to be addressed by the global Plant Health community over the current decade. Euphresco and CIHEAM Bari have concluded that the

inclusive and participatory approach used to strengthen plant health research coordination and transnational collaboration for the Mediterranean region can be used for other regions, and also for global research coordination. Regional consultations may be part of processes that will allow transition from local needs (e.g., reduction of particular pests) to shared global priorities (e.g., prevention of pest spread). Opportunities will be created for scientific communities from less research-intensive countries to reduce their isolation and increase their international exposure. In this context, involvement is essential of organizations and initiatives that are deeply rooted locally and that have leading regional roles. A workshop was organized by Euphresco, Better Border Biosecurity (B3; New Zealand) and the Plant Biosecurity Research Initiative (Australia), and was held on the 20th of September, 2022 in London. This workshop gathered representatives from the Australian Centre for International Agricultural Research (ACIAR), the Centre for Agriculture and Biosciences International (CABI), CIHEAM Bari, the Department for Environment, Food and Rural Affairs (Defra), the Consultative Group on International Agricultural Research (CGIAR), and the National Institute for Agricultural Research and Food Technology, National Research Council (INIA-CSIC). The workshop participants discussed the structures, operations and resources for international research coordination. This started from the models developed in the framework of ongoing Euphresco

⁵ The IPPC, International Plant Protection Convention (<https://www.ippc.int/en/>), is an intergovernmental treaty signed by over 180 countries worldwide that aims to protect the world's plant resources from the spread and introduction of pests, and to promote safer trade.



Figure 2. Transcription and illustration (by Josephine Ford) of the main topics discussed during the Euphresco B3 PBRI workshop, held in London (United Kingdom), on 20 September, 2022.

activities, taking into account the specificities of other international initiatives and organizations (Figure 2). Work will continue throughout 2023, with contributions from policy makers, research funders and research organizations that operate in plant health and are interested in joining this initiative.

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