LIÈGE université
Gembloux
Agro-Bio Tech

Exploring the risks associated with tomato viruses in Belgian diversified production systems and biological characterization of physostegia chlorotic mottle virus.

Coline Temple-Boyer-Dury

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

Exploring the risks associated with tomato viruses in Belgian diversified production systems and biological characterization of physostegia chlorotic mottle virus.

Coline TEMPLE-BOYER-DURY

Dissertation originale présentée (ou essai présenté) en vue de l'obtention du grade de doctorat en sciences agronomiques et ingénierie biologique

Promoteur : Professeur Sébastien Massart
Co - Promoteur : Docteur Arnaud Gérard Blouin

Année civile : 2023

Abstract

Sustainable control of plant diseases is one of the challenges facing agriculture currently. Therefore, it is crucial to understand pathogens in their agroecosystems. Plant viruses represent major pathogens infecting tomato, which is one of the most economically important vegetable worldwide. The impact of virus infection on plants depends on the context, anthropogenic factors and environmental conditions. The constant evolution of viruses through mutations, reassortment or recombination results in the emergence diseases that can potentially threaten crop productivity.

Given the need to improve the sustainability of agriculture, diversified alternative sustainable production systems growing vegetables on soil mainly dedicated to fresh local markets have become more popular in the last decade in industrialized countries such as Belgium, France, or Canada.

This thesis aims to assess and measure the issues associated with tomato viral diseases in diversified Belgian production systems.

First, to achieve this objective, a complementary approach involving socio-economy and plant epidemiology was set up to obtain a more holistic vision of the situation. More specifically, we investigated the tomato virome in 21 diversified vegetable farms, in association with examining the producers' perception, the characteristics of their production systems, and observation of viral-like symptoms in the field. During this survey, one emergent rhabdovirus, physostegia chlorotic mottle virus (PhCMoV), was identified as potential viral threat. This virus was indeed associated with strong symptoms on tomato fruits and in some cases, with a high prevalence in the field. At that time, this virus was only known to infect *Physostegia* and tomato in Austria and Germany and lacked biological information. PhCMoV was associated with the presence of symptoms on tomato fruits.

Thus, the second approach conducted in this thesis to better understand issues related to tomato viral diseases in diversified production systems was to focus on the biological characterization of PhCMoV.

In parallel, informal exchanges with other plant virologists from various research laboratories that simultaneously detected PhCMoV in several European countries and host plants reinforced the interest to focus on its characterization. Thanks to an international collaboration, the research findings of these other research groups on PhCMoV were compiled to gain a comprehensive understanding of the virus' characteristics and associated risks. It enabled to start its characterization regarding its host range, geographical distribution, symptoms causality and genetic diversity. This successful collaboration reports the identification of PhCMoV in nine plant species across seven families and in eight additional European countries. The virus was confirmed to cause severe fruit symptoms on economically important crops such as tomato, eggplant, and cucumber. Phylogenetic analysis showed low genomic variation between samples collected 17 years apart in a same site on two different annual host plants, suggesting strong selection pressure within a specific ecosystem upon time.

Thereafter, other aspects of the virus biology remained to be analyzed, such as transmission mode, incidence and disease severity. A new consortium was created with some research groups already involved in the previous study and new ones, including specialists in entomology and plant virus ecology. In total, eight partners were involved in this second study and experiments were carried out to complete the biological characterization. The natural presence of the disease in multiple sites around our laboratory and the cooperation with the growers were great advantages to study the biology of PhCMoV in its ecosystem. The host range of the virus was expanded to 20 new host plant species, its leafhopper vector (*Anaceratagallia* sp.) was identified, and the observed PhCMoV prevalence in Belgium suggested that the virus is widely spread in the environment. Bioassays in control conditions demonstrated that yield losses can be up to almost 100% if plants are infected at an early stage, while the severity of symptoms decreased with late infection time. The symptoms associated with the virus were also investigated in control conditions on multiple plant species.

To summarize, during this PhD I have explored the risks associated with viruses in soil-grown tomato dedicated to local consumption in Wallonia by developing an innovative methodology that combines grower's perception and virus detection with high throughput sequencing. I have also improved the biological characterization of a newly emergent rhabdovirus (PhCMoV) by collaborating with scientists and setting up field and greenhouse trials, which makes PhCMoV now one of the best characterized newly plant virus. The virus has the potential to be a serious threat on small, diversified farms. Still, the increased knowledge of its biology provided by this work allows initial management measures to be proposed during an outbreak by looking and removing alternative hosts.

Résumé

La lutte durable contre les maladies des plantes est l'un des défis auxquels l'agriculture est actuellement confrontée. Il est donc essentiel de comprendre les pathogènes dans leurs agroécosystèmes. Les phytovirus sont les principaux agents pathogènes qui infectent la tomate, l'un des légumes les plus importants dans le monde sur le plan économique. L'évolution constante des virus par le biais de mutations, de réassortiments ou de recombinaisons entraîne l'apparition de maladies qui peuvent potentiellement menacer la productivité des cultures. L'impact de l'infection virale sur les plantes dépend du contexte, des facteurs anthropogéniques et des conditions environnementales. Compte tenu de la nécessité d'améliorer la durabilité de l'agriculture, des systèmes de production alternatifs diversifiés et durables de culture de légumes sur sol principalement destinés aux marchés locaux sont devenus plus populaires au cours de la dernière décennie dans les pays industrialisés tels que la Belgique, la France ou le Canada.

Cette thèse vise à évaluer et à mesurer les problèmes liés aux maladies virales de la tomate dans les systèmes de production diversifiés belges.

Tout d'abord, pour atteindre cet objectif, une approche complémentaire impliquant la socio-économie et l'épidémiologie végétale a été mise en place afin d'obtenir une vision holistique de la situation. Plus précisément, le virome de la tomate a été étudié dans 21 exploitations maraîchères, tout en examinant la perception des producteurs, leurs systèmes de production et la présence de symptômes de type viral sur le terrain. Au cours de cette étude, un rhabdovirus émergent, le physostegia chlorotic mottle virus (PhCMoV), a été identifié comme une menace virale potentielle. Ce virus était en effet associé à de forts symptômes sur les fruits de tomate et, dans certains cas, à une forte prévalence au champ. À l'époque, ce virus n'était connu uniquement pour infecter le physostegia et la tomate en Autriche et en Allemagne et les informations biologiques manquaient.

Ainsi, la deuxième approche menée dans cette thèse a consistée à se concentrer sur la caractérisation biologique du PhCMoV.

Parallèlement à nos détections en Belgique, des échanges informels avec d'autres experts en virologie végétale provenant de divers laboratoires de recherche ont permis de se rendre compte que le PhCMoV avait simultanément été détecté dans plusieurs pays européens et dans des nouvelles plantes hôtes présentant des symptômes. Cela a renforcé l'intérêt de se concentrer sur sa caractérisation. Grâce à une collaboration internationale, les résultats des recherches de ces huit groupes de recherche sur le PhCMoV ont été compilés afin d'obtenir une compréhension globale du virus et des risques associés. Cela a permis d'entamer sa caractérisation en ce qui concerne sa gamme d'hôtes, sa distribution géographique, la causalité des symptômes et la diversité génétique. Cette collaboration fructueuse fait état de l'identification du PhCMoV dans neuf espèces végétales et sept familles et dans huit pays européens supplémentaires. Il a été confirmé que le virus provoque de graves symptômes sur les fruits de cultures importantes sur le plan économique, telle que la tomate, l'aubergine

et le concombre. L'analyse phylogénétique a montré une faible variation génomique entre des échantillons prélevés à 17 ans d'intervalle sur un même site et sur deux plantes hôtes annuelles différentes, ce qui suggère une forte pression de sélection au sein d'un même écosystème au fil du temps.

Par la suite, d'autres aspects de la biologie du virus restaient à analyser, tels que le mode de transmission, l'incidence et la sévérité. Un nouveau consortium a été créé avec certains groupes de recherche déjà impliqués dans l'étude précédente et de nouveaux, notamment des spécialistes en enthomologie et en écologie virale. Au total, huit partenaires ont été impliqués dans cette deuxième étude et des expériences ont été mises en place pour compléter la caractérisation biologique de ce virus. La présence naturelle de la maladie dans plusieurs sites autour de notre laboratoire et la coopération avec les producteurs a été un grand avantage pour étudier la biologie de ce virus. La gamme d'hôtes du PhCMoV a été étendue à 20 nouvelles plantes hôtes, sa cicadelle vectrice (*Anaceratagallia* sp.) a été identifiée, et la prévalence importante du PhCMoV en Belgique suggère que le virus est largement répandu dans l'environnement. Les bioessais ont montré que les pertes de rendement peuvent atteindre presque 100 % si les plantes sont infectées à un stade précoce, la sévérité de l'impact sur le rendement diminuant avec l'âge de la plante lors de l'infection. Les symptômes associés au virus ont également été étudiés dans des conditions de contrôle sur plusieurs espèces de plantes.

En résumé, au cours de ce doctorat, j'ai exploré les risques associés aux virus dans les tomates cultivées en sol et destinées à la consommation locale en Wallonie en développant une méthodologie innovante qui combine la perception des producteurs et la détection du virus avec le séquençage à haut débit. J'ai également amélioré la caractérisation biologique d'un rhabdovirus émergent en collaborant avec des scientifiques et en mettant en place des essais au champ et en serre, faisant de PhCMoV l'un des nouveaux phytovirus les mieux caractérisés. Ce virus peut constituer une menace sérieuse pour les petites exploitations maraîchères diversifiées. Néanmoins, les connaissances sur sa biologie fournies par ce travail permettent de proposer des mesures préliminaires de gestion lors d'une épidémie comme l'élimination d'hôtes alternatifs.

Acknowledgements

Tout d'abord je souhaite remercier mon directeur de thèse, Sébastien Massart de m'avoir donné l'opportunité de réaliser ce travail en m'accueillant au sein de son équipe. Je lui suis reconnaissante de m'avoir guidée avec expertise tout en me laissant une grande autonomie et la possibilité d'orienter mon sujet de thèse. Je voulais te remercier pour tes bonnes idées, pour ton suivi et pour m'avoir mise en relation avec ton réseau composé de nombreuses personnes ressources. Merci aussi pour ta bonne humeur et ton aide stratégique dans le recentrage de mon travail.

Je souhaite également remercier chaleureusement Arnaud Blouin, mon co-promoteur, qui a joué un rôle essentiel dans mon encadrement et m'a formée dans le domaine de la virologie végétale, que ça soit sur le terrain, en laboratoire ou au bureau. Merci pour avoir partagé ton expertise et avoir répondu à mes innombrables questions quotidiennes avec autant de patience. Nos discussions ont été une immense source de motivation et d'inspiration pour moi et ont fait que je me suis régalée pendant cette thèse. Je souhaite saluer sincèrement la disponibilité que tu m'as accordée tout au long de ce parcours académique qui est essentielle, selon moi, dans la transmission des savoirs et qui m'a permis de me sentir soutenue. Merci aussi pour ton écoute attentive et très humaine.

A vous deux, merci pour vos très bons conseils scientifiques et techniques et votre aiguillage pendant ce travail. Merci pour ses nombreuses réunions et pour vos relectures et commentaires qui ont été indispensables dans mon apprentissage et la réussite de cette thèse.

I express my gratitude to the members of my thesis committee who all have also accepted to be part of my jury for their guidance: Haïssam Jijakli (University of Gembloux), Maja Ravnikar (National Institute of Biology, Slovenia), Ludivine Lassois (University of Gembloux), and Kris De Jonghe (Flanders Research Institute for Agriculture, Fisheries and Food). I would also like to thank Frédéric Francis (University of Gembloux) who accepted to take part to the jury.

I would like to express my gratitude to the Innovative Network for Next generation Training and Sequencing of Virome (INEXTVIR) program for providing us, the 14 phD students who were funded by this EU project, with an ideal international framework for conducting our research. Despite the disruptions caused by the COVID pandemic, working on a collaborative project was highly motivating. I would like to thank Denis Kutnjak for hosting me at their lab in Slovenia and Sophie Tindale for our exchanges on the socio-economic aspect of this project.

I would also like to thank all the co-authors of the articles with whom the collaboration was truly interesting and educational. Especially Eric Verdin from INRAE, Marleen Botermans from NVWA, Stephan Steyer from the CRA-W and all their technicians for setting up bioassays related to study of PhCMoV and Kevin Marechal for the multiple meetings on the socio-economical aspect of this thesis.

Merci à Laurent Minet du centre horticole de Gembloux (CTH) pour son aide précieuse dans la compréhension du PhCMoV sur le terrain. Merci à Claire Olivier du centre interprofessionnel des maraîchers (CIM) pour nos échanges. Enfin, merci aux maraîchers wallons que j'ai interviewés. Merci surtout à ceux chez qui je suis venu des dizaines de fois pour échantillonner et tenter de trouver des cicadelles.

Je voudrais maintenant remercier vivement mes collègues en phytopathologie à Gembloux, pour la dynamique particulièrement motivante et sympathique de l'unité.

En premier mes remerciements s'adressent à mon équipe spéciale « goonies », Nuria et Johan. C'était formidable d'avoir commencé cette aventure au même moment, d'être passé par toutes ses étapes ensemble et d'avoir réellement pu compter les uns sur les autres. Nous nous sommes beaucoup entraidés et amusés et je pense qu'on restera complices longtemps. Merci pour vos nombreux conseils, votre aide dans les analyses bio-informatiques et dans les tâches du quotidien, au laboratoire ou en dehors. Merci pour cet espace de confiance en votre compagnie et ses nombreuses soirées et conférences passées ensembles. Nuria, merci de m'avoir accueilli à Gembloux quasiment une fois par semaine !

Je voudrais aussi remercier François pour nous avoir intégré à l'équipe et pour nous avoir fait autant rire. Merci aussi pour ton soutien sans failles, même depuis les Etats Unis. Nous avons eu une grande chance d'avoir été accompagné par de sacrés Master Goonies pendant nos thèses.

Je voudrais également remercier Fred pour ton aide très importante dans la recherche sur le PhCMoV et pour ton investissement dans la mise en place et la réalisation des essais. Merci pour avoir partagé ton expertise sur les virus et toujours été de très bon conseil. Merci à Gladys pour ton soutien précieux dans toutes les tâches administratives et dans l'organisation des évènements au bureau qui contribuent à la bonne ambiance générale. A vous deux, merci pour votre gentillesse et nos discussions aux pauses du matin.

Merci aussi à Abdoul, Angelo, Bénédicte, Bérénice, Claudia, Cyril, Damien, Gilles, Jimmy, Julien, Igor, Ilhem, Laurena, Lucie, Mathilde, Nikolay, Vanessa et Yves pour votre positivité et bienveillance et tous les bons moments passés ensembles.

Je tiens à remercier ma famille et mes amis pour votre entourage, votre soutien et encouragements. Je voudrais remercier en particulier mes parents pour m'avoir transmis votre intérêt pour la recherche, ma sœur Bertille, mon frère Adrien et mes cousines Marine et Cassie pour leur soutien.

Merci à ma maman pour m'avoir fait aimer la biologie des plantes. Merci à ma cousine Marine pour m'avoir attendue pour notre voyage.

Merci à mes super copines Adèle et Louise pour m'avoir rejointe à la capitale Belge. Ce quotidien avec vous était génial.

Merci à mes ami(e)s du sud, Anaïs, Mathilde, Alexandra, Julien, Elie… avec qui les liens sont restés forts malgré la distance et à ceux que l'on a rencontré plus au nord.

Enfin, un immense merci à mon chéri, Arthur, pour être venu avec moi en Belgique et m'avoir soutenue. Merci de m'avoir fait à manger tant de fois quand je rentrais tard et d'avoir toujours été là pour moi.

Table of content

List of figures

List of tables

List of abbreviations and acronyms

Abbreviations	Definition
ACP	Agroecological crop protection
CIM	Interprofessional center of vegetable growers
CMV	Cucumber mosaic virus
COI	Cytrochrome oxidase I
DNA	Deoxyribonucleic acid
EMDV	Eggplant mottle dwarf virus
ELISA	enzyme-linked immunoassay
EPPO	European and Mediterranean Plant Protection Organization
HTS	High Throuput Sequencing
NCBI	National Center for Biotechnology Information
ORFs	Open reading frames
PCR	Polymerase Chain Reaction
PepMV	Pepino mosaic virus
PVY	Potato virus Y
PYDV	Potato yellow dwarf virus
PhCMoV	Physostegia chlorotic mottle virus
RNA	Ribonucleic acid
RT	Reverse Transcriptase
ToCV	Tomato chlorosis virus
ToMV	Tomato mosaic virus
ToBRFV	Tomato brown rugose fruit virus
TSWV	Tomato spotted wilt virus
TYLCV	Tomato yellow leaf curl virus
VANA	Virion-Associated Nucleic Acids

Chapter 1

Introduction

Preface

Over the past century, the development of intensive agriculture, becoming the predominant agricultural system, enabled a significant increase in crop production. However, the associated use of chemical inputs such as synthetic fertilizers, pesticides, and the need of fossil fuels, as well as the mono-cropping of high-yielding cultivars, resulted in negative impacts on the environment (e.g. soil degradation, biodiversity loss, greenhouse gas emissions, and water contamination) and on human health (e.g. food and water contamination by chemicals and pesticides) (Tilman et al., 2002).

To address these concerns and promote a more sustainable agriculture system, agroecology emerged as a powerful alternative (Kremen et al., 2012, Wezel et al., 2014). This approach promotes the protection of the environment and human health in agriculture and relies on ecosystem services to reach a good productivity (Kremen et al., 2012, Wezel et al., 2014, Ponisio et al., 2015, Hatt et al., 2016, Tamburini et al., 2020). It also promotes inter-disciplinary studies, which can bring together experts from different fields, allowing for a more comprehensive and integrated understanding of the situation, including ecological, social, economic, and cultural factors (Mendez et al., 2013, Hatt et al., 2015).

Small-scale producers of vegetables with agroecological approach, driven by sustainability and ecosystem welfare over profit, are expanding in industrialized countries (Morel and Leger, 2016; Laforge et al., 2018; Dumont et al., 2020). One key aspect of producing vegetables is to sustainably manage plant pests and pathogens which can substantially impact the yield and quality of the products. Accordingly, Agroecological Crop Protection (ACP), for which ecology is the guiding principle, is a powerful approach (Deguine et al., 2023). ACP principle combines the other approaches previously designed to improve the sustainability of crop protection, such as Integrated Pest Management (IPM) or organic agriculture. Still, it is based on a broader and more holistic view of agriculture. IPM focuses on a practical approach to reducing synthetic pesticide use, and organic agriculture is based on strict rules and regulations prohibiting synthetic inputs. ACP is the application of agroecology principles to crop protection, promoting the “one health” approach (Deguine et al., 2023). Its three pillars are: prevention, biodiversity and soil health.

This approach places strong emphasis to the use of knowledge and precise understanding of ecosystems and ecological processes at different scales (plant, field, landscape) in specific contexts alongside socio-economic realities to design, reorganized and implement long-term sustainable farming practices (Deguine et al., 2023). One of its principles is to understand natural interactions better to mimic and use them for crop protection instead of attempting to control and constrain them.

Therefore, in-depth research on the biology, ecology and functioning of pests and pathogens in diversified production systems is crucial to develop and optimizing ACP to improve crop protection and agriculture sustainability alongside maintaining or improving productivity (Kremen et al., 2012).

In this context, I studied the viral diseases of tomatoes in diversified production systems during my PhD thesis and I further characterized an emerging viral disease present in these systems.

Tomato production

Tomato (*Solanum lycopersicum L.*) belongs to the *Solanaceae* family and originated from the Andean region of South America. Its first domestication occurred in Mexico (Benton, 2007). Introduction of tomato in Europe occurred after the mid-16th century. Today, tomatoes are widely cultivated worldwide and are one of the most popular and valuable vegetables, with a gross production of 102.6 billion US dollars and yield estimated at 186.8 million tons (MT) in 2020 (Costa et al., 2018, FAOSTAT, 2020).

Tomatoes can be grown for different purposes, fresh or processed products (sauces, juices…) at various scales, from extensive (small home gardens) to hyper-intensive (large industrial farms). Large-scale commercial production systems often involve high-tech equipment and advanced techniques, while smaller-scale systems may be much simpler and more traditional. In some countries, these systems co exists. For example, in Belgium, where tomato yield was estimated at 0,3 million tons, most of tomato production is focused on export and mass retailing of fresh edible tomato is primarily cultivated in Flanders (the northern part of the country) by specialized tomato growers using high-tech greenhouses. However, small-scale growers who cultivate tomatoes on soil alongside with other vegetables for local consumption and fresh market are also operating in Belgium and their number is increasing (Dumont et al., 2020).

Thousands of different varieties of tomatoes are available, each with unique characteristics that make them suitable for different purposes. Some varieties are better for cooking or canning, while others are ideal for eating fresh. Many different cultivars have been developed to suit different growing conditions, production systems, climates, pest and disease pressures and to reach optimized yields and quality (Chea et al., 2021).

This genotype diversity was created by traditional selection carried out by farmers (Blanca et al., 2022), which is the process of selecting individual plants based on their desired attributes. Modern plant breeding techniques have also contributed to the diversity of tomato varieties by introducing desirable traits from wild species. Tomato production systems are very diverse and can be organized into two main categories: open fields in suitable area (warm and sunny) or under shelters.

1.1 Open field cultivation of tomato

Field production is the traditional and most common method of tomato cultivation, relying on natural sunlight, soil, and rain. Irrigation during dry seasons is common, and mechanization is typically limited to soil preparation. Irrigation and mineral nutrition management must be adjusted based on the soil physical and chemical characteristics. Yields can vary widely from 20 to 100 t/ha, and harvesting practices differ depending on the fruit destination. For example, tomatoes intended for processing are typically mechanically harvested, while those grown for fresh market

are hand-picked over several weeks. Open-field production of tomatoes can be done on a small scale, such as in-home gardens or small family farms, or on a large scale, such as in commercial agriculture operations.

1.2 Under shelter cultivation of tomato

Growing tomatoes under shelter can protected them from unfavorable weather conditions (such as strong winds, heavy rainfall, and extreme temperature) or fluctuations, to which tomatoes are very sensitive. It can also help to prolong the growing season, by protecting plants from cooler temperatures. Overall, the growing conditions can be optimised by creating a controlled environment, resulting in healthier and bigger plants, better-tasting tomatoes and higher yields. Shelters can be heated or unheated.

Tomato culture under cold shelter

Cold shelters can be made of materials such as plastic, glass, or fabric and are used to extend the growing season in cooler climates. Tomatoes grown under cold shelter are mostly grown in soil. To ensure success when growing tomatoes under cold shelter, it is important to monitor the temperature and provide adequate ventilation to prevent humidity buildup and disease problems.

In the south of Spain, particularly in regions like Almeria, tomato production (and horticulture in general) under shelter has been taken to an intensive scale and is mainly dedicated to export. In fact, the concentration of greenhouses is so high that this region is known as the “sea of plastic". On the other hand, in the north of Europe, tomato production under cold shelter is mostly done by small-scale diversified producers, mainly dedicated to fresh local markets.

Overall, the use of shelter for tomato production varies depending on the climate and scale of production. For example, in southern Spain, the intensive use of plastic shelters has enabled year-round production and high yields. In contrast, smaller-scale producers in the north of Europe use shelters to extend the growing season and protect plants from cooler temperatures.

Tomato culture under heaten shelter

Heated shelters make it possible to cultivate tomatoes year-round in colder climates. These shelters are typically glass greenhouses, and the crops are often grown hydroponically using nutrient solutions. The growing conditions are highly controlled, and yields can reach up to 500 tonnes per hectare (eg. Belgium, FAOSTAT 2020). This type of production system requires substantial investment in infrastructure and technology, which are highly sophisticated, making it mainly suitable for industrial-scale tomato production.

Tomato diseases

Tomatoes are highly susceptible to a large range of pests and pathogens which can jeopardize their production. Pests and pathogens infecting tomatoes and their impact on the plants are often inferred to specific climates, geographical locations or production systems and include insects, nematodes, fungi, bacteria, and viruses (Blancard, 2012, Panno et al., 2021 Jeger et al., 2021). Their diversity emphasizes the

importance of the tomato pathosystem as a favorable model for studying plant-pathogen interactions (Arie et al., 2007). In addition, abiotic stress, such as nutrient deficiency or weather extremes, can result in diseased phenotypes and significant yield losses (Blancard, 2012). This section will introduce diseases caused by microorganisms (fungi, oomycetes, bacteria) that can be observed under a microscope. The selected diseases were chosen because they were considered as economically important by Blancard, 2012. Then, the focus of this thesis will be on viruses which requires more specialized equipment to be observed (e.g transmission electron microscope), due to their small size.

1.3 Tomato fungal diseases

Fungi are eukaryotic organisms able to reproduce sexually and asexually. Most pathogenic fungi grow in humid conditions and can cause specific symptoms. Common fungal diseases affecting tomato plants and leading to yield losses include early blight and late blight caused by several species of *Alternaria* (including *A. solani* and *A. tomatophila*) and fungus-like oomycete *Phytophthora infestans*, respectively. These diseases are more frequently observed in tomatoes grown in fields than in greenhouses (Blancard, 2012).

Early blight (Blancard, 2012, Jones et al., 2014, Adhikari et al., 2019)

Early blight (*Alternaria)* mainly affects the aerial part of the plants and is characterized by circular, dark brown spots on leaves and stems and sunken lesions on the fruit with dark concentric rings. In some instances, annual economic yield losses due to this disease have been estimated at nearly 80%. The fungus survives mainly in infected crop residues and soil, but also on the surface of the seeds in the form of conidia and mycelium. In spring, conidia are produced on crop residues, this is the primary infection. Sporulation and spore dispersal are favoured by alternating wet and dry conditions. Conidia are dispersed by wind and water (splash, sprinkler irrigation) on basal leaves or colonized leaves in contact with the soil. *Alternaria* is known only to reproduce asexually. Currently, cultural practices (e.g. rotations, control of humidity, use of healthy seedlings) and fungicide applications are employed for its management due to the lack of resistant cultivars.

Late blight (Blancard et al., 2012, Fry et al., 2015)

Late blight (*P. infestans*) is characterized by presence of greenish black, oily and irregular spots at the apex or at the margin of the old leaves. It can be introduced into an area by infected plants or plant debris, and can also be spread by wind, rain, or contaminated tools and equipments. Late blight develops and spreads rapidly if conditions are humid, rainy and not too warm. The disease can be uncontrolled and cause strong economic damages, especially in field, but also under cold shelter where the conditions are not controlled. Therefore, aeration of tunnels and greenhouses is strongly recommended to reduce the risks of late blight.

P. infestans typically reproduce asexually through sporangia that can be preserved within living tissue. However, when two different strains, such as A1 and A2, come into contact in the field, sexual reproduction may occur as it can be the case in Europe

from the late 1970s (Yuen et Andersson, 2013). Sexual reproduction for *P. infestans* leads to genetic variability, which can increase its aggressiveness, virulence, and resistance emergence to fungicides. Furthermore, oospores produced during sexual reproduction can be particularly resistant to harsh conditions, such as desiccation and cold, making them a more reliable preservation method for the oomycete during winter. In Europe, both strains are present, which complicates disease management.

1.4 Tomato bacteria diseases

Bacteria are prokaryotic organisms which lack true nuclei and other membrane-bound organelles. They reproduce only asexually through binary fission. Bacteria, such as *Ralstonia solanacearum* or *Clavibacter michiganensis* can affect tomato plants and induce diseases. These diseases can be challenging to manage once established and cause significant yield losses, making it essential to implement preventative measures to avoid spreading.

Bacterial wilt disease

Bacterial wilt disease (*Ralstonia solanacearum),* seriously threatens tomato crops, causing significant yield losses and economic damage. This bacteria attacks the plant vascular system and prevents the uptake of water and nutrients. Symptoms of bacterial wilt on tomato plants can include wilting, yellowing and browning of leaves, and eventual death of the plant. In severe cases, annual yield losses due to this disease have been estimated at up to 100%. The disease is particularly prevalent in tropical and subtropical regions, where warm and humid conditions favor the growth and spread of the bacteria. The bacteria responsible for bacterial wilt can survive for many years in the soil and can infect tomato plants through their roots. Once inside the plant, the bacteria multiply rapidly and move up into the xylem, blocking the flow of water and nutrients. The disease is spread through contaminated soil, water, and infected plant material. Unfortunately, no resistant cultivars are currently available to combat bacterial wilt disease in tomatoes. Cultural practices such as crop rotation and healthy seedlings can help reduce the risk of infection, but may not provide adequate protection.

Bacterial canker

Bacterial canker disease is a serious problem for tomato growers worldwide. It is caused by the bacterium *Clavibacter michiganensis* subsp. *michiganensis* and it is characterized by wilting, yellowing, and necrosis of leaves, stems, and fruit. The disease can cause significant yield losses and can be particularly damaging in greenhouses. Bacterial canker is primarily spread through infected seeds, transplants, and plant debris, as well as through mechanical transmission via equipment and tools. The bacteria can survive in the soil for several years, making it difficult to control once established in a field. In addition, there are limited chemical control options, and the development of resistant tomato cultivars has been slow due to the genetic complexity of resistance to the disease. As a result, cultural practices such as crop rotation, sanitation, and use of pathogen-free seed and transplants are critical for managing bacterial canker in tomato production.

Biology: plant virus properties.

1.5 Diversity and virus taxonomy

Plant viruses are microscopic infectious agents capable of replicating only within cells of living organisms, depending on the host cellular machinery. They can be composed of RNA or DNA genomes and are usually encapsidated in a protein coat, known as a "capsid", which protects the genetic information and allows it to infect host cells. The complete structure is called "viral particle". Some viruses are also enveloped by an outer lipoprotein membrane (Hull, 2014).

Plant virus genomes vary in size, structure and complexity (Hull, 2014). Their genome size are mostly in between ~4 and ~20 kb, which ranges amongst the smallest genomes of any organism (Mauck et al., 2018). Some plant viruses have a single-stranded RNA or DNA genome, which can be positive or negative-sense polarity for RNA viruses. In contrast, other species have double-stranded DNA or RNA genomes (Baltimore classification). Virus genomes can either be linear or circular and can be contained in one single nucleic acid molecule (monopartite), several nucleic acid molecules packaged within the same particle (segmented) or into several separate particles (multipartite) (Hull, 2014).

So far, most studies about plant viruses have focused on pathogenic viruses of economically important crops because they can negatively impact food production, food security and economy and because their study requires a lot of resources (Wren et al., 2006, Rybicki, 2015, McLeish and García-Arenal 2020). Nevertheless, the recent development of technologies such as high throughput sequencing (HTS), allowing to detect all (or nearly all) the viruses present in a sample without a priori information has improved the understanding of plant virus diversity (Adams et al., 2018). Traditionally, plant viruses were classified based on a five-rank structure (i.e. species, genus, sub family, family and order). It has recently changed to a 15-rank classification, closely aligned with the Linnaean taxonomic system (Gorbalenya et al., 2020). Nowadays, viruses are classified according to the comparison of their genome sequences of conserved genes and proteins (Gorbalenya et al., 2020, ICTV). The rules which state that a divergent viral sequence is associated with a new viral species is different between plant viral families and are decided by expert groups from the International Committee on Taxonomy of Viruses (ICTV).

Tomato plants are infected by the highest number of viruses among plants to date: at least 312 viral species belonging to 22 families and 39 genera have been recorded to infect it (Figure 1-1, Rivarez et al., 2021). Out of the species listed, there are 220 species of tomato viruses, which have DNA genomes and are divided into three families. The majority of viral species that infect tomatoes belong to the Begomovirus genus (which are DNA viruses) and their associated satellites, due to the high number of species within this genus. In contrast, there is a greater diversity of tomato viruses

among RNA viruses, with 84 known species classified in 18 families (Rivarez et al., 2021).

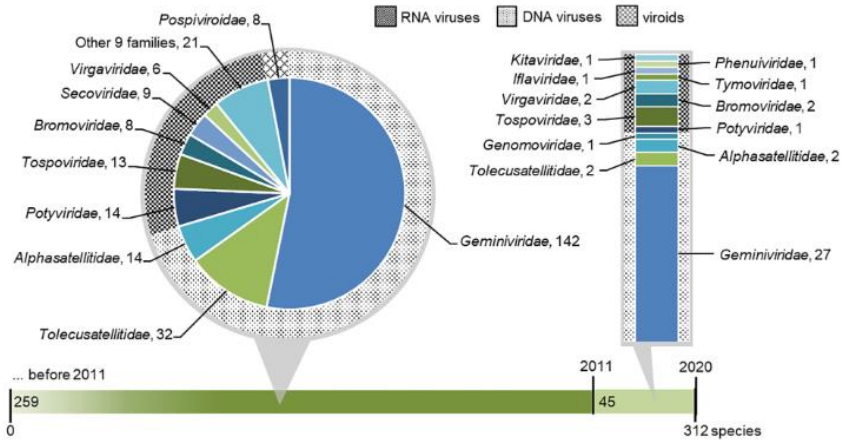


Figure 1-1- Taxonomic distribution of virus and viroid species that were reported to infect or were associated with tomato before 2011 or within the 2011–2020 period (Rivarez et al., 2021)

1.6 Plant virus evolution

Like other viruses, plant viruses evolve through a process called genetic variation and natural selection (Butković et al., 2020). Genetic variation can be represented by genetic errors during virus replication, resulting in changes in their genetic material (mutation, recombination, and reassortment). These genetic changes can be beneficial, harmful, or neutral to the virus (Roossinck et al., 1997). Natural selection then determines which variants of the virus are more adapted to their environment and more likely to survive and spread. Overall, viruses can accumulate genetic changes over short timescales compared to other organisms due to their high mutation rates, large population sizes, and short generation times (Moya et al., 2004). In addition, some type of viruses (eg. RNA viruses) lack proofreading activity in their polymerase proteins and, thus, exhibit the highest mutation rates of any group of organisms (Moya et al., 2004). However, over longer timescales, natural selection slow down the rates of viral evolution which approach those of their hosts (Simmonds et al., 2019). This is because, like their hosts, viruses also experience purifying selection, which removes deleterious mutations that reduce their fitness. As a result, many viral lineages may converge on similar, optimal genotypes that are well-adapted to their host and environment. Furthermore, viruses that are highly specialized to a particular host or environment may experience more limited opportunities for evolution, as they are already well-adapted to their niche (Simmonds et al., 2019). The process of virus evolution through genetic variation and natural selection allows viruses to adapt to changing selection pressures and can result in new viral strains or species with

different biological properties (host range, transmission, severity…) (Figure 1-2). In the context of plant viruses, this can for instance lead to the emergence of new variants or viruses that can overcome the resistance of certain plant varieties or infect a new host. This process contributes to the emergence of plant viral diseases (Elena et al., 2014, McLeish et García-Arenal, 2020).

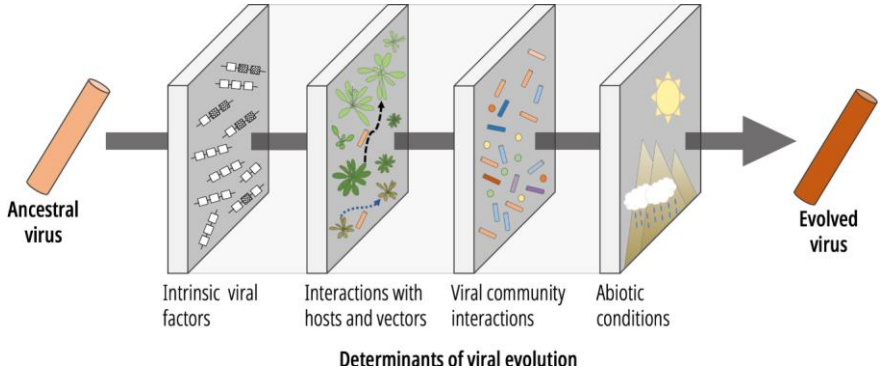


Figure 1-2: Representation of some of the most relevant factors affecting plant virus evolution, from an ancestral to the evolved virus (Butković et al., 2020).

Biology: plant virus characteristics

Tomato viruses and plant viruses can differ in genetic composition and structure but also in their biology and lifecycle, which are crucial to understand for developing sustainable control strategies (Jones et al., 2004). The main biological properties of plant viruses include the host range (i.e. number of plants species infected), the symptomatology and the transmission between plants. These biological properties are closely linked, interdependent, and influenced by environmental factors (Jones and Naidu, 2019). In this chapter, the biological aspects of plant viruses will be explained, illustrated by various tomato viruses, including mainly six selected tomato viruses (Table 1-1). These selected viruses were chosen because they represent well the diversity of biological characteristics of tomato viruses and how they can be managed. Among them, tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV) and cucumber mosaic virus (CMV) are part of the top ten of the most studied and economically important plant viruses (Scholthof et al., 2011). Subsequently (Chapter 5), specific examples will illustrate how knowledge on these biological properties can be used to manage a plant viral disease effectively.

Viral species	Genus	Host range	Transmission main mode	Main symptoms
Tomato mosaic virus (ToMV)	Tobamovirus	145 plants species (27 families)	mechanically (remain stable outside a host), seeds	various (mosaic leaves, leaf distortions, stunted growth, discoloration, marbelling pattern on the fruit…)
Tomato spotted wilt virus (TSWV)	Orthotospovirus	1,000 plants species (85 families)	Thrips (*Frankliniella occidentalis*) (persistent-replicative)	bronzing of the upper sides of young leaves, necrotic spots. Chlorotic spots on rings with concentric (sometimes necrotic) rings
Tomato yellow leafcurl virus (TYLCV)	Begomovirus	49 plants species (16 families)	Whitefly (*Bemisia tabaci*) (persistent- circulative non replicative)	severe stunting, reduction of leaf size, upward cupping/curling of leaves, yellowing of leaves, abortion of flowers
Cucumber mosaic virus (CMV)	Cucumovirus	1,200 plants species (100 families)	Aphids, ∼75 species of aphids (non-persistent)	Expanding leaves typically become twisted, curl downward, and develop a "shoestring" appearance, brown annular spots on fruits
Pepino mosaic virus (PepMV)	Potexvirus	Few Solanaceae sp.	mechanically (remain stable outside a host), seeds	mild interveinal chlorosis (yellowing) and leaf distortions such as spindly leaves
Southern tomato virus (STV)	Amalgavirus	Few Solanaceae sp.	seeds (persistent lifestyle)	no symptoms in single infection

Table 1-1. Six selected tomato viruses with diverse biological properties (Blancard et al., 2012, Hancinský et al., 2020, Tatineni et al., 2023)

Recent advances in metagenomic technologies have expanded our understanding of viruses and their characteristics, unveiling the existence of a significant number of viruses with a persistent life cycle in the environment (Roossinck et al., 2015).

1.7 Host range

The host range is the number of host species that can be infected by a virus (in which a virus can infect, multiply, and be transmitted). It is difficult to assess the natural host range of a virus, but it is commonly accepted that plant host range can vary widely, depending on the virus species and can evolve upon time (Kumar et al., 2020). Some plant viruses have the ability to infect only one or a few closely related plant species (specialists), while others can do so in many different types of plants (generalists) (Kumar et al., 2020). For example, pepino mosaic virus (PepMV) is believed to have a narrow natural host range restricted to species of the *Solanaceae* family. In contrast, cucumber mosaic virus (CMV) or tomato spotted wilt virus (TSWV) can infect more than 1000 different plants species belonging to more than 85 different plant families (Hanssen et al., 2010, Blancard, 2012).

Understanding the host range of plant viruses is essential for plant disease management, as it can help farmers and researchers to develop strategies to prevent or reduce the spread of viruses to susceptible crops (Thresh, 1982, Jones et al., 2004). Most plant viruses cannot survive and remain infectious outside a cell host. Annual crops are absent from the field during winter or dry summer (e.g. tomato), viruses required to infect alternative hosts to survive, called “reservoirs” (Jones et al., 2004). These alternative hosts may be long-lived (perennials) or short-lived with a growing period that overlaps those of crops.

1.8 Transmission

Since most viruses cannot remain stable outside a host, they typically rely on efficient transmission between their host to survive. Plant viruses can be transmitted either vertically, which involves transmission between parents and their progeny; or horizontally, which involves transmission to new plants (Jones and Naidu, 2019).

Horizontal transmission

Horizontal transmission of plant viruses can occur mechanically through arthropods, fungi or nematodes, but described viruses infecting tomatoes are mainly transmitted by insect vectors (Blancard, 2012). The most economically important insect vectors are restricted to a few hemipteran families: aphids, whiteflies, leafhoppers and planthoppers (Hogenhout et al., 2008, Tatineni et al., 2023).

Transmission with insect vector

Transmission mode by insect vectors is usually categorized in four ways depending on: the time the insect needs to feed to acquire the virus (i.e. acquisition phase); (ii) the period between virus acquisition and virus transmission (i.e. latency period); (iii) the time required for virus transmission to a healthy plant (i.e. inoculation phase); and

(iv) time during which the insect can transmit the virus (i.e. retention period) (Bragard et al., 2013, Hull, 2014).

The transmission of plant viruses by insects can take place in a circulatory manner: the virus must circulate in the insect body before it can be transmitted to another plant, and it can replicate in the body of the insect (propagative) or not (non-propagative) or in a non-circulative manner (viruses are retained in the stylet or foregut).

The non-circulative transmitted viruses can either have short acquisition and retention periods in the insects, ranging from a few seconds to a few minutes (non-persistent transmission) to a few minutes or hours (semi-persistent transmission), cannot be transmitted anymore after moulting and do not have latent period. On the other hand, viruses transmitted in a circulative manner have acquisition, latent and retention periods ranging from hours to days or weeks (persistent transmission). The plant viruses which can replicate in their insect vector can be inoculated to healthy plants during the lifespan of the insects and some are transmitted transovarially to the vector progeny (Hogenhout et al., 2008, Hull et al., 2014, Whitfield et al., 2015, Yele et Poddar, 2020). The transmission of insect-transmitted viruses is highly dependent on their vector ecology and the environmental conditions suitable for its development and spread. For example, begomoviruses such as tomato yellow leaf curl viruses (TYLCV) are mainly found in tropical and subtropical area because their vector (whitefly, mainly *Bemisia tabaci*) is well adapted to these climates (Blancard, 2012).

In some cases, it was demonstrated that plant viruses can influence insect behavior by altering the plant chemical composition or its nutritional quality, which can attract or repel insects or by altering the behavior and performances of the insect itself (Eigenbrode et al., 2018). For example, cucumber mosaic virus (CMV), which is transmitted in a non-persistent manner can induce the production of volatile organic compounds in plants which attract aphid vectors, and then, reduces the nutritional quality of the plants for aphids, causing rapid vector dispersal (Mauck et al., 2014, Carmo-Sousa et al., 2014).

Mechanical transmission

Some viruses can also be transmitted mechanically through contact with infected plant debris or contaminated tools, clothes or surface. This transmission mode is less common but can be very efficient notably for pathogenic viruses that infect tomato, making them highly dangerous for crop production. For example, some tobamoviruses (eg. tomato mosaic virus, tomato brown fruit rugose viruses) or potexviruses (pepino mosaic virus) are mechanically transmitted (Panno et al., 2020).

Viruses belonging to these two families (Tobamovirus, Potexvirus) also have special properties which can favor their spread, and the risks associated with their presence, such as being able to remain stable in the environment and remain infectious in the soil, water or any surface for many months (Hull, 2014). For example, tobamoviruses can remain infective even after conventional wastewater treatment (Bačnik et al., 2020).

Vertical transmission

Vertical transmission occur in two ways: through infected seed or infected vegetative propagation material (eg. tubers, grafts). In the case of vegetative propagation of crops (eg. for potato, banana, sweet potatoes, strawberries…), the virus is transmitted when a piece of an infected plant is used to grow a new plant. Vegetative propagation is a highly effective mode of virus transmission and is significant for many viruses that impact crops, given that most viruses can be transmitted through this method.

In the case of an infected seed, the virus is present in the seed coat or within the embryo itself and can be transmitted to the next generation of plants when the seed germinates (Hull, 2014). Viruses vertically transmited are efficiently disseminated worldwide by global trade (Jones et al., 2009).

In tomato, some pathogenic viruses, such as tobamoviruses or potexviruses are transmitted via seeds, but the transmission rate is generally low. For example, Hanssen et al., (2010) showed that pepino mosaic virus (PepMV) can be transmitted at an overall transmission rate of 0.026% under specific experimental condition. However, such low occurrence of seed transmission can be enough to propagate the disease on long distances and to cause an outbreak, if viruses are then easily transmitted in the field, for example, in a mechanical manner (Dombrovsky and Elisheva, 2017, Panno et al., 2020).

Therefore, implementing certification schemes comprising periodic inspections and testing procedures for regulated and quarantined plant viruses before the commercialization and transportation of seeds is essential to avoid the global spread of plant viruses and contain epidemics (Rodoni et al., 2009, Rubio et al., 2021).

1.9 Symptomatology

Plant viruses can cause symptoms in plants by disrupting their physiological and cellular metabolism. Upon infecting a plant, the virus hijacks the plant cellular machinery to replicate itself, which can interfere with the plant normal functions and lead to visible symptoms (Hull et al., 2014). For example, some viruses can interfere with the plant ability to produce chlorophyll, which is necessary for photosynthesis. This can result in yellowing or chlorosis of the leaves, the most common viral symptom (Zhao et al., 2016). In addition, viruses can cause symptoms by triggering the defense mechanisms in plants, which can produce reactive oxygen species and necrotic lesions (dead tissue).

Symptoms caused by viruses are diverse and can be observed in various plant organs (leaves, flowers, fruits). They are general or specific according to the type of virus. A close observation on different indexing plant species allowed to differentiate viruses between each other and to classify them before the use of molecular techniques (Roenhorst et al., 2013). Nevertheless, some viruses induce a large range of symptoms depending on the strains. As a result, different cucumber mosaic virus (CMV) isolates have often been erroneously considered new viral species, when viruses were described only by symptomology (before sequencing time) (Scholthof et al., 2011).

In the field, observing symptoms on plant allows for diagnosing their presence. In tomato, typical symptoms caused by plant viruses includes discoloration of leaves (vein clearing, mosaic, yellowing), leaf deformations (twisted, expanded, curled) and fruits alterations (uneven ripening, stains) (Blancard et al., 2012, Figure 1-3). Virus infection in crops generally leads to reduced tomato fruit production and plant growth (Hull, 2014). These symptoms are general and aspecific as many different viral species belonging to different virus families can cause them. For examples, unvenen ripening of tomato fruits can be observed on tomato infected with PepMV (potexviruses) but also on tomato infected by tobamoviruses (ToMV, ToBRFV), rhabdoviruses (EMDV) etc (Blancard, 2012).

Some symptoms caused by viruses can also be more specific. For example, TYLCV induces stunted plant growth, upward curling of the leaves, yellowing, and flower abortion resulting in a fruit production reduction. TSWV induce concentric necrotic rings on fruits (Blancard, 2012, Figure 1-3).

Figure 1-3 Selected symptoms caused by various important tomato viruses: a) PepMV: uneven ripened fruits (García-Estrada et al., 2022), b) TSWV: concentric and necrotic rings on fruits. (García-Estrada et al., 2022), c) TYLCV: curl upward and interveinal yellowing on leaves (Blancard, 2012), d) CMV: deformed, blistered and mosaic leaves (Blancard, 2012).

It is important to note that the symptoms caused by a same virus vary depending on the different host plant species, cultivars, or even genetically similar plants it infects

(Hull, 2014). Environmental conditions (temperature, luminosity…) and global plant stage and health (nutrient deficiency, or co-infections with other pathogens including other viruses) can alter the expression and severity of symptoms and considerably challenge the diagnosis in the field (Fraile et García-Arenal, 2016, Amari et al., 2021). Overall, viruses fall on a continuum of interactions with its host plant, from mutualism to pathogenic (Gonzalez et al., 2020) (Figure 1-4).

For example, temperature, light intensity, nitrogen and bore concentration in the soil can influence the reaction of tomato plant to ToMV infection (Blancard, 2012). It is also interesting to note that mixed infections of plant viruses are common in nature and there is a gradient of interactions between different viruses in a same host, from synergistic to antagonistic interactions (Syller et al., 2012). A synergistic interaction has a facilitative effect on both, or at least one of the viral partners while an antagonistic type of interaction, only one of the viruses is likely to be the beneficiary, and its presence and activity lower the fitness of the second virus (Syller et al., 2012). These interactions can result in a modification of the symptoms. For example, the symptoms caused by CMV can also be exacerbated in tomato by the presence of another virus such as potato virus Y (Jacquemond, 2012).

In another example, tomato chlorosis virus (ToCV) and TYLCV mixed infections induced synergistic disease effects in tomato plants, resulting in a higher disease severity and growth reduction, a difference of spatio-temporal accumulation of the virus in the different organs of the plants (ToCV accumulated less in upper leaves of ToCV-infected tomato plants than in lower leaves), an overall higher accumulation of the two viruses in the plants. In addition, *B. tabaci* appeared to have a greater TYLCV, but a lower ToCV acquisition rate from mixed infected plants compared with singly infected plants (Li et al., 2021).

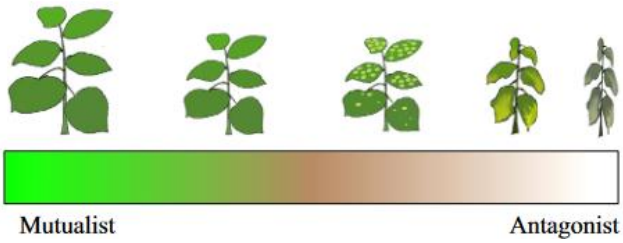


Figure 1-4 Plant viruses on a symbiotic continuum. A virus infected plant may be benefited by virus infection (extreme left), or harmed by the virus to the point of death (extreme right) (Roossinck, 2015)

1.10 Persistent lifestyle of plant viruses in nature

The use and development of technologies such as HTS, revealed that numerous viruses with a persistent lifestyle are prevalent in wild plants (Roossinck et al., 2015,

Maclot et al., 2023). Persistent lifestyle should be distinguished from the persistent modality of transmission (see section 4.2.1.1). Viruses with a persistent lifestyle are transmitted vertically via gametes, do not move between cells within plants and are not associated with apparent symptoms (Roossinck, 2010). Their effect on their hosts is poorly understood. These viruses are members of diverse families (e.g. Amalgaviridae, Endornaviridae, Partitiviridae, Totiviridae) and can infect plants and fungi. For example, one of the most detected persistent lifestyle viruses in tomatoes is the southern tomato virus (STV), a member of the Amalgaviridae family. STV is transmitted by seeds at very high rates (up to 80%) and do not induce symptoms and cell ultra-structural changes in single infection (Elvira-González et al., 2020). It has also been shown that the interactions of STV with other viruses is complex and can result in increase symptoms on tomato plants when co-infection occurred with CMV and PepMV (Elvira-González et al., 2021).

Impact of viruses on plants

,

Plant viruses can significantly impact plants at an ecological and agronomical levels (Jones and Naidu, 2019, Lefeuvre et al., 2019). In both cases, the severity of the impact will depend on the virus biology, its interactions with its host plants and potential vectors and the biology of the vectors. In addition, environmental and human factors can influence the outcome of these interactions (Figure 1-5, Jones and Naidu, 2019, Hančinský et al., 2020).

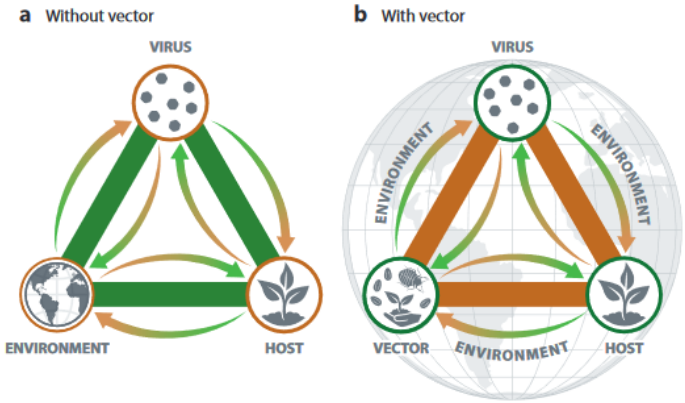


Figure 1-5 Disease triangles for plant virus pathosystems without (a) and with (b) the involvement of virus vectors. Jones and Naidu, 2019

1.11 Ecological impact

Although the ecological role of plant viruses in natural ecosystems is still poorly understood, it is suggested that plant viruses can have significant ecological impacts by affecting the composition and dynamics of plant communities (Roosinck et al,

2015, Maclot et al., 2023), altering plant-herbivore and plant-pollinator interactions (Tack et Dicke, 2013), disrupting ecosystem services, and increasing the risk of plant extinctions (Lefeuvre et al., 2019). These impacts can be complex and far-reaching and have cascading effects on multiple levels of organization and processes within the ecosystem. It is suggested that the "emergence potential" of viruses in natural ecosystems, may play a crucial role in maintaining stability and genetic diversity of natural ecosystems (Lefeuvre et al., 2019). This is particularly important in unmanaged ecosystems, where plant viruses can prevent the overgrowth of genetically homogeneous plants and foster the capacity of ecosystems to endure environmental changes.

1.12 Crop impact

At an agronomic level, viruses can reduce crop yields and alter product quality, affecting food production and the economy (Rybicki et al., 2015, Tatineni and Hein, 2023). The yield losses associated to virus infection are estimated to cost worldwide more than $30 billion annually (Sastry and Zitter, 2014). Viruses can significantly contribute to the reduction of tomato yields, primarily when associated with severe symptoms and efficiently transmitted in a cultivated ecosystem (Nicaise, 2014, Hančinský et al., 2020, Jones et al., 2021, Panno et al., 2021). As a result, viruses are problematic for growers producing tomato at an industrial scale, for example in southeastern Spain where they stand out among other factors of concern such as market fluctuations and production costs (Velasquo et al., 2020). Overall, yield loss due to plant viruses can be mitigated if the pathosystem is well-known and efficient management methods available. In Europe, many studies on tomato viruses are conducted in the Mediterranean basin (where tomato are intensively grown), and 19 viral species were recorded as tomato plant pathogens present in this area (Figure 1-6, Panno et al., 2021).

Viruses	Alfalfa mosaic virus (AMV), Chickpea chlorotic dwarf virus (CpCDV), Cucumber mosaic virus (CMV), Eggplant mottled dwarf virus (EMDV), Parietaria mottle virus (PMoV), Pelargonium zonate spot virus (PZSV), Pepino mosaic virus (PepMV), Potato virus Y (PVY), Southern tomato virus (STV), Tobacco mosaic virus (TMV), Tomato brown rugose fruit virus (ToBRFV), Tomato chlorosis virus (ToCV), Tomato infectious chlorosis virus (TICV), Tomato leaf curl New Delhi virus (ToLCNDV), Tomato mosaic virus (ToMV), Tomato spotted wilt virus (TSWV), Tomato torrado virus (ToTV), Tomato yellow leaf curl virus (TYLCV), Tomato yellow leaf curl Sardinia virus (TYLCSV)	[2,6]

Figure 1-6 List of tomato virus pathogens present in the Mediterranean basin (Panno et al., 2021)

In Belgium, the list of present viruses that can infect tomato includes PepMV, TSWV, ToBRFV, Tomato black ring virus, Impatiens necrotic spot virus, according to the EPPO website. However, this list is likely incomplete because potato virus Y, cucumber mosaic virus (CMV) are also known to be present in Belgium (CABI). A recent large-scale screening of plant viruses using HTS on 18,000 samples of plants within the Solanaceae family in this country (SEVIPLANT project) confirmed the

presence of some of these viruses and also allowed to report new detection in tomato including pathogenic or potentially pathogenic viruses such as tomato chlorosis virus, physostegia chlorotic mottle virus, alfafa mosaic virus, lettuce ring necrosis virus.

In addition, virus's host range evolve and can be broader than what is known. Therefore, we cannot rule out the possibility that some viruses present in Belgium may also have the ability to infect tomatoes, but this is not yet known. Table 1-2 indicates the status of six important tomato viruses in Belgium and their economic impact on crops.

Although the direct impact of plant viruses on yield can be demonstrated under controlled conditions, it is challenging to precisely assess their impact in the field due to multiple co-factors interplaying with the different components of the pathosystems such as plant health, infection timing, the efficiency of virus spread, presence of mixed infections, cultural practices, weather... In addition, the economic impact of pathogenic viruses on crops depends on the production systems and the crop value. For example, the economic impact of PepMV on the tomato industry has been strongly debated, as the impact largely depends on the structure of the tomato market, more specifically on the marketability and economic value of lower-quality fruits, which differs considerably between growing areas (Spence et al., 2006, Hanssen et al., 2010). Nevertheless, some studies have estimated the economic losses caused by specific viruses. For example, in the Dominican Republic, TYLCV outbreaks were estimated to have caused losses exceeding 10 million US dollars (Gilbertson et al., 2007). In another study conduced in Samsun province (9 474 km2), Turkey, aiming to determine the effect of TSWV on tomato yield components under field conditions, the estimated yield losses due to TSWV was around 0.9 million US dollars per year for outdoor tomato production (Sevik et Arli-Sokmen, 2012).

The risks of developing one or more viral diseases resulting in yield losses vary depending on the production systems, the environmental conditions and presence of pathogenic viruses around the fields. For example, monoculture tomato fields can be more susceptible to viral infections, as all plants have the same genetic makeup and are equally vulnerable to a symptom-causing viruses (Jones et al., 2004). On the other hand, if the conditions are favorable for the spread of a viral disease in a region, (e.g. ideal temperature for the reproduction of insects vectors) the risks of disease development will be higher.

In addition, when farms are located in a same region, or belonging to a same production system, the likelihood of virus spread within the system or area can increase due to frequent connections. This is particularly true in southern Spain, where horticultural production is concentrated in a small area (Panno et al., 2021). For example, workers moving between neighbouring farms can transmit viruses from one farm to another. Moreover, if a virus is efficiently transmitted by a particular variety of seeds that growers commonly utilize, it can readily propagate to farms that obtain their seeds from the same suppliers (Velasco et al., 2020).

The fact that production systems impact viral epidemic can also be illustrated by the case of PepMV: the risk of developing the disease can be higher in glasshouse

hydroponic production systems where plants are grown in nutrient solutions without soil, as the virus can easily spread through water and irrigation systems (Blancard, 2012). Furthermore, the effectiveness of management techniques aimed at mitigating yield losses caused by plant viruses may not be universally applicable, and as a result, the development and impact of plant viral diseases would be once again dependent on the specific production system. For instance, insect control measures are considerably more successful in tightly controlled, high-tech greenhouses compared to open field production systems. Consequently, viruses transmitted by insects are less likely to affect tomato yields in high-tech greenhouses with strict control measures than those grown in open fields.

Viral species	Current economic importance	Disease distribution	Statut in Belgium
ToMV	Moderate, mitigate by resistant genes	worlwide	Present, no details
TSWV	High	worlwide	Present, restricted distribution (A2 list)
TYLCV	High	Tropical and subtropical regions of the world	Absent, confirmed by survey (A2 list)
CMV	Moderate	worlwide	Present, no details
PepMV	Moderate, mitigate by cross protection	worldwide (glasshouse)	Present, restricted distribution (A2 list)
STV	Low (persistent lifestyle)	worlwide	Present, no details

Table 1-2. Six selected tomato viruses and their global economic impact, distribution and status in Belgium (Blancard et al., 2012, Hancinský et al., 2020, Tatineni et al., 2023)

Agronomy: plant virus management

No curative treatment exists for plant viruses. Therefore, the negative impact of a virus on crops can be minimized by applying different management practices tailored to the specific virus causing the disease and its unique biological properties (Jones et al., 2004). Some practices can be applied upstream to avoid virus introduction, while others can reduce virus spread. The earlier pathogenic plant viruses are identified, the more effective control strategies can be. Surveillance are, therefore critical in plant virus management (Miller et al., 2009, Pluess et al., 2012, MacDiarmid et al., 2013, Mumford et al., 2016, Jeger et al., 2021).

Certain tomato viruses which significantly impact plant production may be subject to regulation by national or continental authorities. There are different levels of surveillance, and the virus regulation can evolve based on how the virus spreads and on the policy of countries/ political contexts. In Europe, the European and Mediterranean Plant Protection Organization (EPPO) recommends its member countries to regulate quarantine pests and pathogens listed in two lists. A1 list includes pests and pathogens absent from the EPPO region. A2 list includes pests and pathogens which are locally present in the EPPO region. In Europe, tomato viruses in the A1 list include tomato mottle virus. Tomato viruses that are in the A2 lists include PepMV, tomato brown rugose fruit virus, tomato chlorosis virus, tomato infectious chlorosis virus, tomato ringspot virus, TSWV, tomato leaf curl New Delhi virus TYLCV and related begomoviruses (EPPO).

In Belgium, the Federal agency for the safety of the food chain (FASFC) oversees setting the rules and applying them regarding quarantine schemes. It follows the European regulation and EPPO recommendations. The most important aspects of the legislation concern “phytosanitary import and export controls” and “plant passports”.

The designation “quarantine pathogen” entails specific restrictions and regulations designed to prevent the virus's introduction and spread within a given geographical region where it should not be present. Such measures may include mandatory testing and certification procedures for imported or transported plants, regular monitoring, and sanitary precautions (Rodoni et al., 2009, Jones et al., 2021). Regular control in nurseries is necessary to limit the spread of infected plants in non-contaminated area (Jones et al., 2009).

1.13 Virus detection

Virus detection is crucial for plant virus management to identify the causal agent of a viral disease and to monitor their presence for further disease management (Miller et al., 2009, Mumford et al., 2016, Rubio et al., 2020).

Field observations

Identifying the plant virus(es) causing the observed disease is the first step in developing an effective control strategy in the field (Jones et al., 2009, Rubio et al., 2019). Viral symptoms can be confused with abiotic stress, such as nutrient deficiency, phytotoxicity, or other pathogens such as bacteria (Blancard, 2012). Therefore, identifying the causal agent of disease symptoms requires a lot of expertise and knowledge in multiple domains and gathering information on the cultural context and environmental conditions. For instance, it is necessary to know the prevalent pathogens in the studied region, to have information on their biology, the symptoms they typically cause on different host plants, and how the crop is supposed to grow normally (MacDiarmid et al., 2013, Mastin et al., 2022). For example, the presence of insects at the start of the season, or the cultural practices (e.g. phytosanitary products use, rotations, sensitive or resistant cultivars use…) can also aid in determining the cause of the disease (Blancard, 2012).

Identifying a plant viral disease in the field starts with observing the symptoms and their distribution on the different organs on the plant (fruits, flowers, leaves…) and to compare it with healthy looking plants (if possible). The distribution of the symptomatic plants in the parcel can also provide a clue about the cause of the disease or type of virus (Blancard, 2012). If plants display symptom that resemble a viral symptom (e.g. yellowing) in a field in patchy distributions, it is more likely that an insect-transmitted virus (e.g. aphids) is involved in the disease transmission than a mechanically transmitted virus (Blancard, 2012).

In some cases, field observations, appropriate knowledge and expertise can result in a confident diagnosis. However, misidentification can also occur. Plant viruses are challenging to identify with certainty at the species level as multiple viral species can induce similar symptoms and plants are often co-infected by several plant viruses, which can impact the expression of symptoms (Panno et al., 2021, Moreno et al., 2020).

Laboratory-based tests

Laboratory testing is therefore often crucial to confirm and determine the specific virus responsible for a disease, especially when the disease pose a threat to production and necessitate to be managed. Additionally, in situations where the impact of the disease could be significant, laboratory testing can provide an accurate identification of the causal virus and better understanding of the extent of the problem, enabling taking the most relevant decision for dealing with the disease.

Laboratory test of plant viruses can be used to assess presence of virus in a preventive manner to avoid the introduction or spread of a virus (e.g. screen of planting material before it is introduced into a new area through screening of quarantine pathogens) (Rodoni, 2009, MacDiarmid et al., 2013, Rubio et al., 2020).

An extensive range of laboratory tests is available to validate the presence or absence of plant viruses. The most common tests are based on the protocols of Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR), which were respectively developed in the 1970s' and 1980s' (Clark and Adams, 1977, Vunsh et al., 1990). These tests are generally hypothesis-driven, meaning that the protocol will depend on the virus(es) suspected to cause the disease. Recently, high throughput put sequencing (HTS) techniques combined with bioinformatics tools were developed to assess the presence of multiple plant viruses in a sample without a priori knowledge of it (Kreuze et al., 2009, Massart et al.,2014, Maclot et al., 2020, Lebas et al., 2022). These technologies revolutionized the field of plant virus research, diagnostic and management.

Each technique has its own advantages and drawbacks, and the best diagnostic method for plant viruses depends on the specific goals of the analysis, the available resources and time. The basic principles of the different type of tests are:

- ELISA is a serological test used to detect plant viruses by measuring the presence of viral antigens in plant extracts (Clark and Adams, 1977). The test uses antibodies that specifically bind to the viral antigens. Monoclonal antibodies are generated by merging a myeloma cell with a single clone of B-

cells, and they specifically target a distinct epitope, which is a small segment of an antigen. Polyclonal antibodies, on the other hand, are produced from multiple clones of B-cells and recognize multiple epitopes on the same antigen. Then, the presence of the virus is visualized through the use of a labeled secondary antibody. ELISA is a simple, rapid, and relatively inexpensive diagnostic test broadly used for routine surveillance of known viruses.

- Polymerase Chain Reaction (PCR) is a molecular biology technique that amplifies specific DNA sequences from a sample (Vunsh et al., 1990). In the case of plant virus detection, a sample of plant tissue is first processed to extract the viral DNA or RNA. If the virus is encoded by a RNA genome, an additional retrotranscription (RT) step, which transforms RNA into complementary DNA (cDNA) is necessary. Then, specific primers (DNA sequences pieces designed to hybridize the targeted DNA sequence specifically) amplify a portion of the viral genome. This test is highly specific and sensitive and can detect even small amounts of viruses (Vunsh et al., 1990).

- HTS method allows the detection without a priori of all the viruses present in a sample and the generation of their complete or full genome sequences by simultaneous massive sequencing of nucleic acids (RNA or DNA) extracted from infected plant tissues (Massart et al., 2014). The viral sequences are then analyzed using bioinformatic tools and compared to databases of known viral sequences to identify the viruses infecting the sample. This method does not require a priori knowledge on the type of virus present in the sample, which considerably facilitates the detection of new plant viral species and surveillance studies (Massart et al., 2014, Adams et al., 2018, Villamor et al., 2019) and which supports plant trade by being able to declare the material free from pathogens (Maree et al. 2018).

1.14 Management strategies

Viral disease control relies mainly on prophylactic measures to restrain virus introduction or dispersion or, when possible, on plant resistance (Rubio et al., 2020). It is strongly depending on a good understanding of the pathosystem (the relationship between the virus, its host plants, its vector (if existing) and the environment) in specific production system (Jones et al., 2004, Nicaise, 2014). The choice and implementation of a strategy depends on the specific virus involved, the severity of the disease, the unique characteristics of the crop and environment and production system in which they are grown (Jones et al., 2004). It will also strongly depend on the economic or environmental cost vs. the yield benefit and its feasibility (to which extent the practice can be used in different production systems or countries). The main type, characteristics and drawbacks of control strategies are described below.

Use of virus-free planting materials

Using certified planting materials can reduce the risk of introducing viral infections and prevent the spread of viruses to healthy plants (Jeger et al., 2021). Therefore, phytosanitary certification systems based on diagnostic tests with high sensitivity and specificity have been established worldwide to certify the propagation of regulated plant viruses (Rubio et al., 2020). Network of diagnostics laboratories and phytopathologists such as EPPO facilitates the standardization of protocol and procedures and the detection of regulated plant viruses before commercialization as mentioned before (Miller et al., 2009, MacLeod et al., 2016, EPPO).

On another hand, it is possible to obtain planting materials free from certain viruses for propagative crops through virus sanitation following various methods, including tissue culture, meristem culture, and heat therapy. In cases where viruses are seed-coat transmitted, disinfection of seed by treatments with hydrochloric acid, trisodium phosphate, or sodium hypochlorite can be applied to eliminate viruses on the seed surface and prevents virus transmission (Davino et al., 2020).

Sanitation practices

To limit the transmission of viruses from one year to another, or from one crop to another, it is possible to reduce or eliminate them in the environment, within and around the field. The strategies used depend on the biology of the virus. For example, for viruses that can remain infectious in the environment (e.g. tobamoviruses, potexviruses), it is possible to disinfect surfaces and equipment that come into contact with the plants to kill the viruses (greenhouses surfaces, clothes, tools…). Thorough cleaning is always very laborious but is much more feasible and practicable in high-tech hydroponic tomato greenhouses than in field crops (e.g. complicated to decontaminate soil). Protocols and phytosanitary procedures exist for different types of pathogens in multiple production systems, especially for regulated viruses (EPPO, European union law (commission implementing regulation (EU) 2019/2072, MacDiarmid et al., 2013, Kumar et al., 2021).

For viruses that cannot remain active without a host and take refuge, in alternative host plants (eg. perennial plants, weeds), identifying and eliminating these plants may help to reduce their presence (Jones et al., 2004, Blancard, 2012, Lecoq et al., 2013).

Sanitation practices can also be implemented throughout the growing season, to minimize the transmission of the virus to non-infected plants and its accumulation in the environment. For instance, regular disinfection of tools can prevent the spread of viruses to healthy plants. In addition, removal of infected plants and weeds, which could serve as a reservoir for further transmission, can prevent the buildup of viruses in or around the parcel and prevent from secondary infection.

Control of insect-transmitted viruses

Various strategies can be employed to limit the viral epidemics by targeting the insect vectors . The goal is to reduce the vector population or prevent plants from coming into contact with the insects. It can include the use of insecticides, biological control (using natural enemies of the insect vectors), and physical barriers such as shelters or nets (Jones et al., 2009).

Nevertheless, some of the virus/vector control strategies are not sustainable and can be costly. For example, applying chemicals for insect control might be harmfull to non-targeted insects and have severe consequences on the pollinator's population, ecosystems, environment and human health (Ansari et al., 2014, Nicolopoulou et al., 2016). It is therefore, important to use them judiciously (eg. when the plants are young and more sensitive to plant viruses). In addition, using insecticides on non-persistent plant viruses is useless because the virus is transmitted so quickly that the insecticide cannot effectively prevent transmission (Blancard et al., 2012).

When a single control strategy is overused for many years at a large scale, viruses or vectors can adapt to the constraints, and the control methods will become ineffective (Jones et al., 2004). For example, the targeted insect vector of TYLCV, *Bemisia tabaci*, developed resistance against insecticides which were intensively used for its control, resulting in a critical TYLCV epidemic (Jones et al., 2004, Horowitz et al., 2005).

Ultimately, a combination of approaches is generally more successful to effectively manage plant viruses transmitted by insects while minimizing the impact on non-targeted insects and the environment.

Use of resistant plant varieties

Plant resistance, obtained through breeding or genetic engineering, is one of the most effective strategies for controlling viruses in plants, as it reduces the likelihood of infection (Jones et al., 2004, Rubio et al., 2019, Tatineni and Hein 2023). However, obtaining stable virus resistance is time-consuming and complicated, requiring a challenging screening process that utilizes complex genetics. As a result, resistant varieties are limited in availability for some pathogenic tomato viruses (ToMV, TSWV, TYLCV). When specific resistant varieties are available, they can be extremely useful in limiting damage caused by viruses. For example, the use of resistant varieties with dominant resistance genes (Tm-1, Tm-2 and Tm-22) that have been introgressed from wild tomatoes to cultivated ones and have considerably reduced serious losses caused by ToMV and tobacco mosaic virus (TMV) worldwide (Hanssen et al., 2010).

However, plant viruses can overcome plant resistance through evolution (Ciuffo et al., 2005, Rubio et al., 2019). Nevertheless, breaking down plant resistance can involves a tradeoff leading to a loss of the virus fitness in non-resistant hosts, thus limiting the cases of emergence and spread of resistance-breaking isolates in the field (García-Arenal and McDonald, 2003, Rubio et al., 2019).

On the other hand, some resistance can also be broken by changed environmental conditions. For example, the single resistance gene against TSWV confered by Tsw is temperature sensitive and fails to function at or above 32 °C (De ronde et al., 2019).

Vaccine cross protection.

Cross-protection involves pre-treating plants with a mild or attenuated strain of the virus to protect them from more severe or virulent strains of the virus. This process triggers a defense response in the plant that primes it against subsequent infection by the more virulent strain of the virus (Ziebell et Caar, 2010). Cross-protection has been

used successfully to control PepMV in tomato (Hanssen et al., 2009, Schenk et al., 2010, Vermunt et Kaarsemaker, 2017, Pechinger et al., 2019). This method is believed to be promising for controlling plant viruses in the future (Pechinger et al., 2019). However, it is important to note that cross-protection must be used under controlled and scientific conditions. Inappropriate use of cross-protection techniques without proper understanding or control, can lead to unintended consequences and potentially dangerous outcomes.

Integrated disease management

To conclude, multiple examples described above showed that when a single control strategy is overused for many years at a large scale, it is likely that viruses or vectors adapt to the constraints, and the control methods will become ineffective (Jones et al., 2004). As a result, combining multiple approaches to fight against viruses with available host resistance, cultural, chemical, and biological control measures which is described as "integrated disease management" (IDM) is recommended (Jones et al., 2004, Jones and Naidu, 2019, Velasco et al., 2020). Nevertheless, not all the practices are applicable or efficient in different production systems or countries. For example, the use of insectides is banned from organic agriculture.

The emergence of plant virus challenges their management.

Overall, management strategies aim to balance the economical impact viruses can have on crops. They strongly relies on a good understanding of the pathosystem (Jones and Naidu, 2019). Therefore, one of the biggest challenge of plant virus control is the continuous evolution and emergence of plant viruses and their vectors which have new biological properties and can overcome designed strategies to control previously existing diseases triggering the need to adapt the control measures (Hanssen et al., 2009, Velasco et al., 2020, Ristaino et al., 2021). Viruses have been reported to cause almost half of the emergent infectious plant diseases (Anderson et al., 2004). Emergent viral diseases can be due to known viruses which suddenly become epidemics in new contexts (host, country, production system, climatic conditions…), the geographic spread of a known virus in new locations or new viruses or variant that result from natural evolution and selection (see Chap3.2, Hanssen et al., 2009, Jones et al., 2009, Jones and Naidu, 2019). The emergence of plant viral diseases is driven by various anthropogenic and natural factors which increase instability, such as trade (e.g. exchange of plant material which can favor new encounters between plants and viruses), agricultural practices (e.g. monoculture which can favor the adaptation of pathogens to new host) and climate change (Jones et al., 2009, Elena et al., 2014, Roossinck et Garcia- Arenal, 2015, Tatineni et al., 2023). Early detection of emergent plant viral diseases and rapid response are crucial to compensate for these risks (Miller et al., 2009, Pluess et al., 2012, MacDiarmid et al., 2013, Mumford et al., 2016). The earlier a new disease is identified, the more effective can be the strategies to limit its spread in a country or field. The development of HTS technologies has revolutionized the surveillance of plant viral diseases, including emergent ones (Massart et al., 2014,

Olmos et al., 2018, Villamor et al., 2021). However, in many cases, efficient biological characterization of new viruses is needed to understand the associated risks and to adapt or set up management strategies (MacDiarmid et al., 2013, Massart et al., 2017, Adams et al., 2018).

1.15 Study case: biological characterization of ToBRFV

For example, since less than a decade, the emergent disease caused by ToBRFV significantly alerted the tomato industry and all the involved stakeholders. This virus overcame the resistance gene Tm-22, the most effective way of controlling tobamoviruses and, thus, triggered a cascade of studies (Luria et al., 2017, Zhang et al., 2022). For more context, ToBRFV was identified for the first time from glasshouse tomato plants grown in Jordan (Salem et al., 2016). The plants showed mild foliar symptoms at the end of the season, strong brown rugose symptoms on fruits, and disease incidence was close to 100 % (Salem et al., 2016). The authors reconstructed the whole genome sequence and studied its phylogenetic relationships amongst the other tobamoviruses to assess the novelty of this virus species. Tobamoviruses are well-known for the extrem stability of their virion, which can remain viable for months to years outside of their host plant and can spread very efficiently through direct contact (Creager et al., 1999, Velasco et al., 2020, Panno et al., 2021). Subsequently, the virus was identified in Israel (Luria et al., 2017), where the authors conducted a comprehensive molecular, biological and epidemiological characterization. They confirmed Koch's postulates for the disease, validating the causal association with severe fruit symptoms, followed by partial host range determination showing that capsicum and some weeds can also be infected. They revealed that ToBRFV overcame the resistance against tobamoviruses. Subsequently, ToBRFV occurrence was reported in various locations in all over the world while countries were developing and implementing appropriate quarantine and phytosanitary measures to limit the spread (Okaldun et al., 2019). Many research efforts were conducted, mobilizing a lot of resources and all the latest available technologies to develop efficient detection methods, to understand the disease in its globality (all the transmission pathways of the virus, its distribution, host range, stability…) and to set preventives measures (elimination of infected plants and debris, disinfection of seeds, tools and greenhouses, crop rotation…). Its pest risk assessment was established very quickly compare to other novel plant viruses due to its high economic importance (Rivarez et al., 2021). A strong part of the research was also dedicated to developing resistant varieties, which is the best way of controlling tobamoviruses and some varieties are already available (Oladokun et al., 2019, Zhang et al., 2022).

In Flanders (northern part of Belgium) and the Netherlands, it has been reported in the newspapers that some growers tried to protect their crops using homemade, not controlled cross-protection. This practice is illegal as it can be very dangerous for tomato production (increases the virus pressure within a farm, which challenges its elimination in a site and increases the risk that neighboring farms will become infected with the virus)

1.16 How to address the detection of new viruses

Most of the time, new viruses can not trigger all the research efforts and ressources that were dedicated to ToBRFV to characterize and to assess the threat for crop production (Hou et al., 2020, Rivarez et al., 2021). Indeed, since the HTS technologies and bioinformatics tools have become widespread in plant virus studies, the number of newly discovered viruses has increased at an unseen rate (Massart et al., 2014). These technologies were used to instantly describe ToBRFV as a new viral species and then, to detect it in various countries and host plants. During the period 2011-2020, the use of HTS in several studies allowed the detection of 14 new viral species infecting tomato which was already one of the best characterize crops in its ability to host plant viruses (Rivarez et al., 2021). Among these viruses, tree viruses (tomato brown rugose fruit virus (ToBRFV), tomato mottle mosaic virus (ToMMV) and tomato necrotic stunt virus (ToNStV) were likely to induce important diseases in tomatoes and were further characterized (Rivarez et al., 2021). However, research on most viruses were not followed up after the initial discovery and first publication (Rivarez et al., 2021).

Genomic sequences generated with HTS and metadata can assist the assessment of the risk to crop production. For example, if a novel virus belongs to a well-described family or genera known to be associated with a pathogenic virus or if it was isolated in single infection from a symptomatic economically crop, it will require more attention than if it is not associated with symptoms on a wild plant (Fontdevila et al., 2023).

Still, the information do not allow to assess with certainty the risk a new virus can pose to the production, to detect it and to manage it properly. This requires experimental confirmation which is a resource and time-consuming process (study on the host range, transmission, symptomatology, severity, incidence… see Figure 1-7) (Massart et al., 2017, Adams et al., 2018). This process, which is essential to manage plant viruses can not follow the pace of virus discovery (Massart et al., 2017, Hou et al., 2020). The review of Rivarez et al., 2021 highlights that even for viruses which infect an economical important crop such as tomato, biological characterization is most of the time, not completed after the first discovery.

To address this bottleneck and to help scientist to consciously judge which new viral species necessitates to be characterized for assessing the risk they can pose to the production and at what point, a scaled and progressive scientific framework was proposed in 2017 (Massart et al.,. 2017) and updated due to the increased rate of virus discovery and the parallel evolution of technological tools that can help improving the characterization (Fontdevila et al., 2023, see Figure 1-7). These guidelines include regular exchanges with stakeholders (scientific, local authorities, advisers, institutions, growers…) at strategic moment to assess whether a novel virus must be considered as a phytosanitary priority or not and would require continuing into the characterization process. The risk a virus can pose for the production is therefore re-evaluated with stakeholders after critical time points in the characterization. The

framework also guides scientists to prioritize, optimize and accelerate the characterization of the growing number of new viruses according to the available tools and through prepublication data sharing. It also emphasizes reconsidering causal association between symptoms and virus presence and to focus on experimental evidence, the strength of the relationship, consistency of the relationship, and a binary evaluation of coherence and plausibility as described by Fox in 2020. Overall, Rivarez et al., (2021) emphasized that completeness of virus characterization greatly depended on the phytosanitary priority level of a virus.

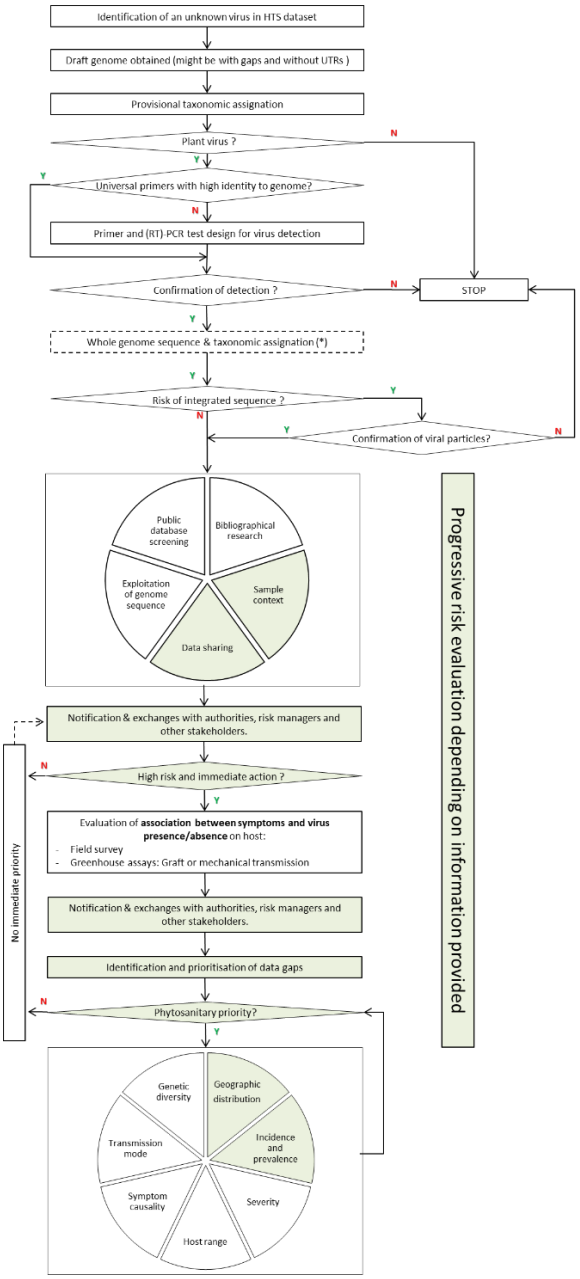


Figure 1-7. Proposed framework following the discovery of a novel virus or viroid. Y means positive response (yes) and N means negative response (no). Multi-stakeholders are involved in green-highlighted actions, and researchers in white-highlighted actions. Fontdevila et al., 2023.

Inter disciplinary integration for improving plant pathogen studies.

Pathogen management can benefit from trans/inter-disciplinary approach that incorporates knowledge from different stakeholders (eg. growers, advisors, scientific) and from various type of experts in different disciplines (socio-economy, ecology, biology…) because it can bring together diverse perspectives and expertise to have a more realistic and holistic overview of the situation's complexity (Breukers et al., 2012, Mauser et al., 2013, Mumford et al., 2016, Jeger et al., 2021, Jones et al., 2021, Ristaino et al, 2020, Deguine et al., 2023). Interdisciplinary research combines methods and perspectives of different disciplines to address the same research question or problem in a coherent ensemble. Transdisciplinary research involves collaboration with stakeholders outside of academia (e.g. growers) in the research process through a reflective processes that seek to integrate different knowledge systems (Mauser et al., 2013, Fernández González et al., 2021).

Research in agroecology and Agroecological Crop Protection (ACP) explicitly promotes integrative and cooperative studies such as inter/trans disciplinary studies since sustainable pathogen risk management requires a comprehensive understanding of the complex interactions between ecological, social, and economical factors in agricultural systems. This approach acknowledges that no single discipline or stakeholder group can fully understand or address the challenges of sustainable pathogen risk management (Fernández González, 2021, Deguine et al., 2023). In addition, when producers and scientists collaborate in a research process, they should both directly take advantages of the outcome of the research (Fernández González, 2021, Iocola et al., 2022, Deguine et al., 2023).

It is also recommended to integrate knowledge, methods, and expertise from different disciplines ("convergence science") at multiple scales (from the molecular to the landscape level) in order to better understand and tackle emerging plant diseases and improve plant biosecurity (Miller et al., 2009, Mumford et al., 2016, Rostinio et al., 2020).

Nevertheless, research in plant virology is rarely conducted alongside (or put into perspectives) other disciplines, such as social sciences or alongside growers' perception (Breukers et al., 2012, Jeger et al., 2021). For example, many studies on tomato plant viral diseases were conducted but the perception of producers regarding these diseases was never considered (Xu et al., 2017, Desbiez et al., 2020, Li et al., 2021, Rivarez et al., 2022).

Growers are the first to be affected by the damage a virus can cause and are also the ones who can impact its epidemiology at field and landscape scales. They have direct and frequent inspections of the plants, and their observations of symptoms and disease evolution, combined with their field expertise and practice, can be valuable in determining if a viral disease (new or not) represents a threat and should therefore be a priority for further research (Deguine et al., 2023). Since viruses can affect crops differently depending on environmental conditions and contexts, growers' expertise

can also provide valuable empirical information, bring clues on the occurrence and emergence of a new viral disease and help to better understand it (Miller et al., 2009). In addition, the management measures taken or envisioned by producers vary considerably among them, depending on production systems (Breukers et al., 2012). It is, therefore, valuable to be in touch with the realities of the producers to adapt virus research to their needs and beliefs in relation to different production systems.

Technical advisers and extension services can play a crucial role in communication between scientific communities. On one hand, they provide information and technical assistance to farmers regarding how to manage plant diseases and on another hand, they can help to monitor plant diseases (emergent or not) and to communicate important information (e.g. presence of emergent diseases) with the scientist community and help to ensure the accuracy of scientific research and findings (Miller et al., 2009). Nevertheless, these extension services are underdeveloped in developing countries (Carvajal-Yepes et al., 2019, Ristaino et al., 2020) and not all producers can afford to pay for their services, particularly those operating with limited economic resources (Cuéllar et al., 2011).

Each production systems have its own particularities, which vary between countries, socio-economical and cultural contexts. Producers' decisions (actions or inactions) are related to their specific production systems, past experience, motivations, knowledge and perception and the global regulations of the country. They can accelerate or slow down, the dispersion of plant viral diseases (Breukers et al., 2012, Murray-Watson et al., 2022). Human action and agricultural practices can also enhance the emergence of plant virus diseases (Thresh, 1992, Anderson et al., 2004, Jones et al., 2009, Jones and Naidu, 2019). In addition, monitoring (surveillance) of plant diseases by national and regional plant protection agencies, which depends on politics and economics of nations is crucial in dealing with emergent plant diseases (Carvajal-Yepes et al., 2019, Ristaino et al., 2020).

Therefore, having a holistic view of the specific system and context, and considering the perception and awareness of producers and other stakeholders regarding plant viruses could help to understand their spread, introduction and survival strategies and to improve their surveillance (Miller et al., 2009, Mumford et al., 2016, Rostinio et al., 2020, Jones and Naidu, 2019, Deguine et al., 2023). This approach can also help scientists to design appropriate research directions tailored to a particular context and providing support to producers in managing plant viral diseases accurately according to their production systems and to the socio-economic context (Wilkinson et al., 2011, Breukers et al., 2012, Hatt et al., 2018, Hong et al., 2020, Jeger et al., 2021, Deguine et al., 2023). Indeed, if grower perception is added to scientific knowledge, and if contextualization of plant diseases is better assessed, it may help to evaluate what are the priorities for managing existing or new plant viral disease (more communication, more general or specific knowledge on a pathosystem…) (He et al., 2016, Deguine et al., 2023).

For example, several studies in developing countries focus on how grower's perceive tomato viral diseases challenging their production and response to it. They highlighted that growers can lack knowledge on how to manage a plant virus epidemic

in their fields because of a lack of basic biological information (such as host range or transmission mode). As a consequence, inappropriate and inefficient management strategies were applied such as the application of fungicides on insect-transmitted viruses (e.g. on begomovirus) (Nagaraju et al., 2002, Schreinemachers et al., 2015, Islam et al., 2017).

Therefore, we suggested that conducting an epidemiological virus survey while studying farmers' perceptions and production systems alongside applying high-tech tools and complex protocols for virus detection may enhance the understanding of viruses comprehensively and accurately. This, in turn, can be appreciated by farmers as they would be taken into consideration in the process and might result from direct benefices (eg. a better understanding of the diseases present in their field).

Study context:

In the south part of Belgium (Wallonia), agriculture historically strongly relies on cereals and livestock. However, in recent years, there has been a noticeable rise in the number of small-scale vegetable growers who sustainably cultivate a wide diversity of vegetables for the fresh consumption and local market (Figure 1-8). This trend is not limited to Wallonia alone but has also been observed at the national level in Flanders, and at an international level in countries such as France, England, and Canada. The profession is becoming more popular and attractive (Dumont et al., 2017).

In 2017, the number of producers growing vegetables for the fresh market was estimated at 364 in Wallonia (Dumont, 2020). Nearly half was supervised by the walloon extension services (Center interprofessional of vegetables producers (CIM)).

In a sociological study, Dumont classified the different vegetable production systems for the fresh market according to their technical orientation (size of the farm, motorization, average area developed per vegetable...) and production model (conventional, organic, agroecology). She showed that producers who grow a large diversity of vegetables (25-50 different plant species) on small (<2.5 ha) and medium (<10 ha) areas in organic farming can be considered as oriented towards an agroecological approach. In addition, the vast majority of vegetable growers in Wallonia are small-scale producers who are agroecologically oriented (Dumont et Barrett, 2017). These production systems are nowadays encouraged by Wallonia government, which, within the common agricultural policy framework of the 2023-2027, provides an annual premium of 4,000 € per ha on the first three hectares of organic diversified vegetable farms, at least for farms of less than ten hectares in total.

By studying the sociological profile of these small-scale vegetable producers and their motivations, the thesis of Dumont showed that many producers without farming background are attracted by this profession and set up, motivated by ethical and environmental considerations which can drive their choices in term of cultural management.

Their production system can be considered "alternative" as it does not rely on conventional vegetable production principles. Regarding pathogen management, producers engaged in an agroecological approach often adopt practices that promote soil health, biodiversity, and natural pest management (crop rotation, intercropping, biological control…). By promoting a diverse and healthy ecosystem, they aim to reduce the incidence and severity of plant diseases on their global production without relying heavily on synthetic pesticides which makes their approach more preventive and resilient than curative (Wezel et al., 2014, Deguine et al., 2023). Cultivating a wide range of vegetables, can also allow producers to be more resilient and to better follow their ecological principles. For example, they can have more flexibility to avoid chemical treatments to “save” a crop in case it is affected by a disease as they can rely on other crops instead. Biodiversity provides an insurance, or a buffer, against environmental fluctuations because different species respond differently to change (Lin, 2011). It is also recognized that diversifying the production of cultivated plants can result in many agronomical benefices (“ecosystem services”) such as enhancing pest control, pollination, soil fertility, etc., which improve the sustainability of production without compromising crop yield (Wezel et al., 2014, Ponisio et al., 2015, van der Ploeg et al., 2019, Pepin et al., 2021, Tamburini et al., 2020).

Nevertheless, cultivating a wide range of vegetables can imply different behavior to manage the culture and may lead to new disease risks. For example, many rare tomato varieties that are not necessarily recommended by the main technical research centers can be grown in diversified production systems, which increases the diversity of the systems but may favor some diseases as they lack resistance genes (Hanssen et al., 2009). Another potential risk is that producers may import plant material through various means, such as online purchases of exotic plant species or exchanges, in order to provide a wide selection of unique and original vegetables to their customers. However, these methods may not be closely regulated and could increase the likelihood of introducing harmful pathogens (e.g. quarantine pathogens, Fox et al., 2018). Therefore, these more sustainable systems might be likely to be threatened by different dynamics of disease or by different diseases than those known for conventional and large-scale systems. In addition, diseases present in these systems need to be studied and characterized because, in absence of pesticide use, producers aim to rely heavily on preventive measures and therefore, on a good understanding of plants and pathogens biology and ecology. The problems linked to plant viruses in these emergent production systems are currently unknown.

Furthermore, the outdoor production of various vegetables in Belgium (and in this particular cold climate) is a recent development, and climate change is already underway. Since shifts in agricultural practices and environmental conditions can lead to the emergence of new plant viral diseases (Jones et al., 2009), which can rapidly spread if not detected early, the need to understand them in such new systems is even higher.

Figure 1-8 Diversified production system. Tomatoes are grown under the shelters during summer.

Objectives of the thesis

My PhD goal is to better understand the risks associated with viral diseases in Belgium tomatoes grown in soil within diversified production systems for the local fresh market. More specifically, the first objective is:

I- To explore the risks caused by plant viruses on tomato grown in an emergent production system where unsustainable control methods would not easily be adopted.

To reach this goal, I have developed an innovative survey, which includes (i) the perception of 21 growers regarding plant viral diseases, (ii) field observations for viral disease symptoms and (iii) virus detection by applying HTS technologies and bioinformatics analyses.

Whether certain factors can explain the risks associated with the detected plant viruses in the different farms was also investigated through the second main goal of this thesis which is:

II- To improve the biological characterization of a new viral disease identified in these systems and caused by Physostegia chlorotic mottle virus (PhCMoV).

PhCMoV was simultaneously detected in multiple European countries (Belgium, Germany, France, Serbia, Slovenia…) on tomato showing severe fruits deformations and anomalies of coloration. Therefore, its biology was first studied thanks to an international cooperation between different scientists and prepublication data sharing. This work has led to fill knowledge gaps related to the genetic diversity of the virus, its historical and geographical distribution, its host range and symptomatology.

Then, bioassays and field survey were performed to complete the missing knowledge gaps related to its incidence, transmission and severity. This work was undertaken because it is crucial to improve knowledge on these biological aspects to assess the risks a new virus can pose for the production and because the virus was identified during the first part of the thesis as problematic.

References

Adams, I. P., Fox, A., Boonham, N., Massart, S., and De Jonghe, K. 2018. The impact of high throughput sequencing on plant health diagnostics. Eur J Plant Pathol. 152:909–919.

Adhikari, P., Oh, Y., and Panthee, D. R. 2017. Current Status of Early Blight Resistance in Tomato: An Update. International Journal of Molecular Sciences. 18:2019.

Amari, K., Huang, C., and Heinlein, M. 2021. Potential Impact of Global Warming on Virus Propagation in Infected Plants and Agricultural Productivity. Frontiers in Plant Science. 12.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., and Daszak, P. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology & Evolution. 19:535–544.

Ansari, M. S., Moraiet, M. A., and Ahmad, S. 2014. Insecticides: Impact on the Environment and Human Health. In *Environmental Deterioration and Human Health: Natural and anthropogenic determinants*, eds. Abdul Malik, Elisabeth Grohmann, and Rais Akhtar. Dordrecht: Springer Netherlands, p. 99–123.

Bačnik, K., Kutnjak, D., Pecman, A., Mehle, N., Tušek Žnidarič, M., Gutiérrez Aguirre, I., et al. 2020. Viromics and infectivity analysis reveal the release of infective plant viruses from wastewater into the environment. Water Res. 177:115628.

Blanca, J., Pons, C., Montero-Pau, J., Sanchez-Matarredona, D., Ziarsolo, P., Fontanet, L., et al. 2022. European traditional tomatoes galore: a result of farmers' selection of a few diversity-rich loci. Journal of Experimental Botany. 73:3431–3445.

Blancard, D., Les maladies de la tomate - Identifier, connaître, maîtriser. Quae : des livres au coeur des sciences. Librairie Quae.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J. J., MacFarlane, S., Peters, D., et al. 2013. Status and prospects of plant virus control through interference with vector transmission. Annu Rev Phytopathol. 51:177–201.

Breukers, A., van Asseldonk, M., Bremmer, J., and Beekman, V. 2012. Understanding Growers' Decisions to Manage Invasive Pathogens at the Farm Level. Phytopathology®. 102:609–619.

Butković, A., and González, R. 2022. A brief view of factors that affect plant virus evolution. Frontiers in Virology. 2.

Carmo-Sousa, M., Moreno, A., Garzo, E., and Fereres, A. 2014. A non-persistently transmitted-virus induces a pull-push strategy in its aphid vector to optimize transmission and spread. Virus Res. 186:38–46.

Carvajal-Yepes, M., Cardwell, K., Nelson, A., Garrett, K. A., Giovani, B., Saunders, D. G. O., et al. 2019. A global surveillance system for crop diseases. Science. 364:1237–1239.

Chea, L., Erika, C., Naumann, M., Smit, I., Horneburg, B., and Pawelzik, E. 2021. Morphological, Leaf Nutrient, and Fruit Quality Characteristics of Diverse Tomato Cultivars under Organic Low-Input Management. Sustainability. 13:12326.

Ciuffo, M., Finetti-Sialer, M. M., Gallitelli, D., and Turina, M. 2005. First report in Italy of a resistance-breaking strain of Tomato spotted wilt virus infecting tomato cultivars carrying the Sw5 resistance gene. Plant Pathology. 54:564–564.

Clark, M. F., and Adams, A. N. Y. 1977. Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses. Journal of General Virology. 34:475–483.

Costa, J. M., and Heuvelink, E. 2018. The global tomato industry. Tomatoes. :1–26.

Creager, A. N. H., Scholthof, K.-B. G., Citovsky, V., and Scholthof, H. B. 1999. Tobacco Mosaic Virus: Pioneering Research for a Century. The Plant Cell. 11:301–308.

Cuéllar-Padilla, M., and Calle-Collado, Á. 2011. Can we find solutions with people? Participatory action research with small organic producers in Andalusia. Journal of Rural Studies. 27:372–383.

Davino, S., Caruso, A. G., Bertacca, S., Barone, S., and Panno, S. 2020. Tomato Brown Rugose Fruit Virus: Seed Transmission Rate and Efficacy of Different Seed Disinfection Treatments. Plants. 9:1615.

Deguine, J.-P., Aubertot, J.-N., Bellon, S., Côte, F., Lauri, P.-E., Lescourret, F., et al. 2023. Agroecological crop protection for sustainable agriculture. In *Advances in Agronomy*, Academic Press.

Desbiez, C., Wipf-Scheibel, C., Millot, P., Berthier, K., Girardot, G., Gognalons, P., et al. 2020. Distribution and evolution of the major viruses infecting cucurbitaceous and solanaceous crops in the French Mediterranean area. Virus Research. 286:198042.

Dumont, A. M., and Baret, P. 2017. *Classifications et cartographies des systèmes de production et commercialisation des légumes frais en Région wallonne (Belgique)*.

Dumont, A. M., Gasselin, P., and Baret, P. V. 2020. Transitions in agriculture: Three frameworks highlighting coexistence between a new agroecological configuration and an old, organic and conventional configuration of vegetable production in Wallonia (Belgium). Geoforum. 108:98–109.

Eigenbrode, S., Bosque-Pérez, N., and Davis, T. 2018. Insect-Borne Plant Pathogens and Their Vectors: Ecology, Evolution, and Complex Interactions. Annual Review of Entomology. 63.

Elena, S. F., Fraile, A., and García-Arenal, F. 2014. Chapter Three - Evolution and Emergence of Plant Viruses. In *Advances in Virus Research*, eds. Karl Maramorosch and Frederick A. Murphy. Academic Press, p. 161–191.

Elvira González, L., Peiró, R., Rubio, L., and Galipienso, L. 2021. Persistent Southern Tomato Virus (STV) Interacts with Cucumber Mosaic and/or Pepino Mosaic Virus in Mixed-Infections Modifying Plant Symptoms, Viral Titer and Small RNA Accumulation. Microorganisms. 9:689.

Elvira-González, L., Medina, V., Rubio, L., and Galipienso, L. 2020. The persistent southern tomato virus modifies miRNA expression without inducing symptoms and cell ultra-structural changes. Eur J Plant Pathol. 156:615–622.

Fernández González, C., Ollivier, G., and Bellon, S. 2021. Transdisciplinarity in agroecology: practices and perspectives in Europe. Agroecology and Sustainable Food Systems. 45:523–550.

Fox, A. 2020. Reconsidering causal association in plant virology. Plant Pathology. 69:956–961.

Fox, A., Fowkes, A. R., Skelton, A., Harju, V., Buxton-Kirk, A., Kelly, M., et al. 2019. Using high-throughput sequencing in support of a plant health outbreak reveals novel viruses in Ullucus tuberosus (Basellaceae). Plant Pathology. 68:576–587.

Fraile, A., and García-Arenal, F. 2016. Environment and evolution modulate plant virus pathogenesis. Curr Opin Virol. 17:50–56.

Frontiers | Application of HTS for Routine Plant Virus Diagnostics: State of the Art and Challenges.

Fry, W. E., Birch, P. R. J., Judelson, H. S., Grünwald, N. J., Danies, G., Everts, K. L., et al. 2015. Five Reasons to Consider Phytophthora infestans a Reemerging Pathogen. Phytopathology®. 105:966–981.

García-Arenal, F., and McDonald, B. A. 2003. An analysis of the durability of resistance to plant viruses. Phytopathology. 93:941–952.

García-Estrada, R., Diaz-Lara, A., Aguilar-Molina, V., and Tovar-Pedraza, J. 2022. Viruses of Economic Impact on Tomato Crops in Mexico: From Diagnosis to Management—A Review. Viruses. 2022:1251.

Gilbertson, R., Rojas, M., and Jaquez, J. 2007. Introduction of Tomato Yellow Leaf Curl Virus into the Dominican Republic: the Development of a Successful Integrated Pest Management Strategy. Tomato Yellow Leaf Curl Virus Disease. 279–303.

González, R., Butković, A., Escaray, F. J., Martínez-Latorre, J., Melero, Í., Pérez-Parets, E., et al. 2021. Plant virus evolution under strong drought conditions results in a transition from parasitism to mutualism. Proceedings of the National Academy of Sciences. 118:e2020990118.

Gorbalenya, A. E., Krupovic, M., Mushegian, A., Kropinski, A. M., Siddell, S. G., Varsani, A., et al. 2020. The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks. Nat Microbiol. 5:668–674.

Hančinský, R., Mihálik, D., Mrkvová, M., Candresse, T., and Glasa, M. 2020. Plant Viruses Infecting Solanaceae Family Members in the Cultivated and Wild Environments: A Review. Plants. 9:667.

Hanssen, I. M., Lapidot, M., and Thomma, B. P. H. J. 2010a. Emerging viral diseases of tomato crops. Mol. Plant Microbe Interact. 23:539–548.

Hanssen, I. M., Mumford, R., Blystad, D.-R., Cortez, I., Hasiów-Jaroszewska, B., Hristova, D., et al. 2010b. Seed transmission of Pepino mosaic virus in tomato. Eur J Plant Pathol. 126:145–152.

HANSSEN, I. M., and THOMMA, B. P. H. J. 2009. Pepino mosaic virus: a successful pathogen that rapidly evolved from emerging to endemic in tomato crops. Mol Plant Pathol. 11:179–189.

Hatt*, S., Artru*, S., Brédart, D., Lassois, L., Francis, F., Haubruge, É., et al. 2016. Towards sustainable food systems: the concept of agroecology and how it questions current research practices. A review. Biotechnol. Agron. Soc. Environ.

Hatt, S., Boeraeve, F., Artru, S., Dufrêne, M., and Francis, F. 2018. Spatial diversification of agroecosystems to enhance biological control and other regulating services: An agroecological perspective. Science of The Total Environment. 621:600–611.

He, D., Zhan, J., and Xie, L. 2016. Problems, challenges and future of plant disease management: from an ecological point of view. Journal of Integrative Agriculture. 15:705–715.

Hogenhout, S. A., Ammar, E.-D., Whitfield, A. E., and Redinbaugh, M. G. 2008. Insect Vector Interactions with Persistently Transmitted Viruses. Annual Review of Phytopathology. 46:327–359.

Hong, Q. N., Rees, R., Sutcliffe, K., and Thomas, J. 2020. Variations of mixed methods reviews approaches: A case study. Research Synthesis Methods. 11:795–811.

Horowitz, A., Denholm, I., and Morin, S. 2007. Resistance to Insecticides in the TYLCV vector, Bemisia Tabaci. In *Tomato Yellow Leaf Curl Virus Disease*, , p. 305–325.

Hou, W., Li, S., and Massart, S. 2020. Is There a “Biological Desert” With the Discovery of New Plant Viruses? A Retrospective Analysis for New Fruit Tree Viruses. Front. Microbiol. 11.

Hull, R. 2014a. Chapter 1 - Introduction. In *Plant Virology (Fifth Edition)*, ed. Roger Hull. Boston: Academic Press, p. 3–14.

Hull, R. 2014b. Preface. In *Plant Virology (Fifth Edition)*, ed. Roger Hull. Boston: Academic Press, p. vii–viii.

Iocola, I., Angevin, F., Bockstaller, C., Catarino, R., Curran, M., Messéan, A., et al. 2020. An Actor-Oriented Multi-Criteria Assessment Framework to Support a Transition towards Sustainable Agricultural Systems Based on Crop Diversification. Sustainability. 12:5434.

Islam, W. Management of Plant Virus Diseases; Farmer’s Knowledge and our Suggestions. Hosts and Viruses. :7.

Jacquemond, M. 2012. Chapter 13 - Cucumber Mosaic Virus. In *Advances in Virus Research*, Viruses and Virus Diseases of Vegetables in the Mediterranean Basin, eds. Gad Loebenstein and Hervé Lecoq. Academic Press, p. 439–504.

Jeger, M., Beresford, R., Bock, C., Brown, N., Fox, A., Newton, A., et al. 2021. Global challenges facing plant pathology: multidisciplinary approaches to meet the food security and environmental challenges in the mid-twenty-first century. CABI Agriculture and Bioscience. 2:20.

Jones, R. A. C. 2009. Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Research. 141:113–130.

Jones, R. A. C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Research. 100:5–30.

Jones, R. A. C., and Naidu, R. A. 2019. Global Dimensions of Plant Virus Diseases: Current Status and Future Perspectives. Annual Review of Virology. 6:387–409.

Jr, B. 2007. *Tomato Plant Culture: In the Field, Greenhouse, and Home Garden, Second Edition*.

Kremen, C., Iles, A., and Bacon, C. 2012. Diversified Farming Systems: An Agroecological, Systems-based Alternative to Modern Industrial Agriculture. Ecology and Society. 17.

Kreuze, J. F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., et al. 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. Virology. 388:1–7.

Kumar, M., Bharti, R., and Ranjan, T. 2020. The Evolutionary Significance of Generalist Viruses with Special Emphasis on Plant Viruses and their Hosts. The Open Virology Journal. 14.

Kumar, P. L., Cuervo, M., Kreuze, J. F., Muller, G., Kulkarni, G., Kumari, S. G., et al. 2021. Phytosanitary Interventions for Safe Global Germplasm Exchange and the Prevention of Transboundary Pest Spread: The Role of CGIAR Germplasm Health Units. Plants (Basel). 10:328.

Laforge, J., Fenton, A., Lavalée-Picard, V., and McLachlan, S. 2018. New farmers and food policies in Canada. Canadian Food Studies / La Revue canadienne des études sur l’alimentation. 5:128–152.

Lebas, B., Adams, I., Al Rwahnih, M., Baeyen, S., Bilodeau, G. J., Blouin, A. G., et al. Facilitating the adoption of high-throughput sequencing technologies as a plant pest diagnostic test in laboratories: A step-by-step description. EPPO Bulletin. n/a

Lefeuvre, P., Martin, D. P., Elena, S. F., Shepherd, D. N., Roumagnac, P., and Varsani, A. 2019. Evolution and ecology of plant viruses. Nature Reviews Microbiology. 17:632–644.

Li, J., Wang, J., Ding, T., and Chu, D. 2021a. Synergistic Effects of a Tomato chlorosis virus and Tomato yellow leaf curl virus Mixed Infection on Host Tomato Plants and the Whitefly Vector. Front. Plant Sci. 12.

Li, Y., Tan, G., Xiao, L., Zhou, W., Lan, P., Chen, X., et al. 2021b. A Multiyear Survey and Identification of Pepper- and Tomato-Infecting Viruses in Yunnan Province, China. Frontiers in Microbiology. 12.

Lin, B. B. 2011. Resilience in Agriculture through Crop Diversification: Adaptive Management for Environmental Change. BioScience. 61:183–193.

Long-Term Anthropogenic Management and Associated Loss of Plant Diversity Deeply Impact Virome Richness and Composition of Poaceae Communities | Microbiology Spectrum.

Luria, N., Smith, E., Reingold, V., Bekelman, I., Lapidot, M., Levin, I., et al. 2017. A New Israeli Tobamovirus Isolate Infects Tomato Plants Harboring Tm-22 Resistance Genes. PLOS ONE. 12:e0170429.

MacDiarmid, R., Rodoni, B., Melcher, U., Ochoa-Corona, F., and Roossinck, M. 2013. Biosecurity Implications of New Technology and Discovery in Plant Virus Research. PLOS Pathogens. 9:e1003337.

MacLeod, A., Jones, G. D., Anderson, H. M., and Mumford, R. A. 2016. Plant health and food security, linking science, economics, policy and industry. Food Sec. 8:17–25.

Maclot, F., Candresse, T., Filloux, D., Malmstrom, C. M., Roumagnac, P., van der Vlugt, R., et al. 2020. Illuminating an Ecological Blackbox: Using High Throughput Sequencing to Characterize the Plant Virome Across Scales. Front. Microbiol. 11.

Management of Virus and Viroid Diseases of Crops in the Tropics | SpringerLink.

Massart, S., Candresse, T., Gil, J., Lacomme, C., Predajna, L., Ravnikar, M., et al. 2017. A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies. Front. Microbiol. 8.

Massart, S., Olmos, A., Jijakli, H., and Candresse, T. 2014. Current impact and future directions of high throughput sequencing in plant virus diagnostics. Virus Research. 188:90–96.

Mastin, A. J., van den Bosch, F., Bourhis, Y., and Parnell, S. 2022. Epidemiologically-based strategies for the detection of emerging plant pathogens. Sci Rep. 12:10972.

Mauck, K. E., Chesnais, Q., and Shapiro, L. R. 2018. Chapter Seven - Evolutionary Determinants of Host and Vector Manipulation by Plant Viruses. In *Advances in Virus Research*, Environmental Virology and Virus Ecology, ed. Carolyn M. Malmstrom. Academic Press, p. 189–250.

Mauck, K. E., De Moraes, C. M., and Mescher, M. C. 2014. Biochemical and physiological mechanisms underlying effects of Cucumber mosaic virus on host-plant traits that mediate transmission by aphid vectors. Plant Cell Environ. 37:1427–1439.

Mauser, W., Klepper, G., Rice, M., Schmalzbauer, B. S., Hackmann, H., Leemans, R., et al. 2013. Transdisciplinary global change research: the co-creation of knowledge for sustainability. Current Opinion in Environmental Sustainability. 5:420–431.

McLeish, M. J., Fraile, A., and García-Arenal, F. 2020. Trends and gaps in forecasting plant virus disease risk. Annals of Applied Biology. 176:102–108.

Méndez, V. E., Bacon, C. M., and Cohen, R. 2013. Agroecology as a Transdisciplinary, Participatory, and Action-Oriented Approach. Agroecology and Sustainable Food Systems. 37:3–18.

Miller, S. A., Beed, F. D., and Harmon, C. L. 2009. Plant Disease Diagnostic Capabilities and Networks. Annual Review of Phytopathology. 47:15–38.

Morel, K., and Léger, F. 2016. A Conceptual Framework for Alternative Farmers' Strategic Choices: The Case of French Organic Market Gardening Microfarms. Agroecology and Sustainable Food Systems. 40.

Moreno, A. B., and López-Moya, J. J. 2020. When Viruses Play Team Sports: Mixed Infections in Plants. Phytopathology. 110:29–48.

Moya, A., Holmes, E. C., and González-Candelas, F. 2004. The population genetics and evolutionary epidemiology of RNA viruses. Nat Rev Microbiol. 2:279–288.

Mumford, R. A., Macarthur, R., and Boonham, N. 2016. The role and challenges of new diagnostic technology in plant biosecurity. Food Sec. 8:103–109.

Murray-Watson, R. E., Hamelin, F. M., and Cunniffe, N. J. 2022. How growers make decisions impacts plant disease control. PLOS Computational Biology. 18:e1010309.

Nagaraju, N., Venkatesh, H. M., Warburton, H., Muniyappa, V., Chancellor, T. C. B., and Colvin, J. 2002. Farmers' perceptions and practices for managing tomato leaf curl virus disease in southern India. International Journal of Pest Management. 48:333–338.

Nicaise, V. 2014. Crop immunity against viruses: outcomes and future challenges. Frontiers in Plant Science. 5.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., and Hens, L. 2016. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Frontiers in Public Health. 4.

Oladokun, J. O., Halabi, M. H., Barua, P., and Nath, P. D. 2019. Tomato brown rugose fruit disease: current distribution, knowledge and future prospects. Plant Pathology. 68:1579–1586.

Olmos, A., Boonham, N., Candresse, T., Gentit, P., Giovani, B., Kutnjak, D., et al. 2018. High-throughput sequencing technologies for plant pest diagnosis: challenges and opportunities. EPPO Bulletin. 48:219–224.

Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandić, A., et al. 2021. A Review of the Most Common and Economically Important Diseases That Undermine the Cultivation of Tomato Crop in the Mediterranean Basin. Agronomy. 11:2188.

Pechinger, K., Chooi, K. M., MacDiarmid, R. M., Harper, S. J., and Ziebell, H. 2019. A New Era for Mild Strain Cross-Protection. Viruses. 11:670.

Pépin, A., Morel, K., and van der Werf, H. M. G. 2021. Conventionalised vs. agroecological practices on organic vegetable farms: Investigating the influence of farm structure in a bifurcation perspective. Agricultural Systems. 190:103129.

van der Ploeg, J. D., Barjolle, D., Bruil, J., Brunori, G., Costa Madureira, L. M., Dessein, J., et al. 2019. The economic potential of agroecology: Empirical evidence from Europe. Journal of Rural Studies. 71:46–61.

Pluess, T., Jarošík, V., Pyšek, P., Cannon, R., Pergl, J., Breukers, A., et al. 2012. Which Factors Affect the Success or Failure of Eradication Campaigns against Alien Species? PLOS ONE. 7:e48157.

Ponisio, L. C., M'Gonigle, L. K., Mace, K. C., Palomino, J., de Valpine, P., and Kremen, C. 2015. Diversification practices reduce organic to conventional yield gap. Proceedings of the Royal Society B: Biological Sciences. 282:20141396.

Ristaino, J. B., Anderson, P. K., Bebber, D. P., Brauman, K. A., Cunniffe, N. J., Fedoroff, N. V., et al. 2021. The persistent threat of emerging plant disease pandemics to global food security. Proc Natl Acad Sci U S A. 118:e2022239118.

Rivarez, M. P. S., Vučurović, A., Mehle, N., Ravnikar, M., and Kutnjak, D. 2021. Global Advances in Tomato Virome Research: Current Status and the Impact of High-Throughput Sequencing. Front. Microbiol. 12.

Rodoni, B. 2009. The role of plant biosecurity in preventing and controlling emerging plant virus disease epidemics. Virus Research. 141:150–157.

Roenhorst, J. W., Botermans, M., and Verhoeven, J. T. J. 2013. Quality control in bioassays used in screening for plant viruses. EPPO Bulletin. 43:244–249.

de Ronde, D., Lohuis, D., and Kormelink, R. 2019. Identification and characterization of a new class of Tomato spotted wilt virus isolates that break Tsw-based resistance in a temperature-dependent manner. Plant Pathology. 68:60–71.

Roossinck, M. J. 2010. Lifestyles of plant viruses. Philosophical Transactions of the Royal Society B: Biological Sciences. 365:1899–1905.

Roossinck, M. J. 1997. Mechanisms of Plantvirus Evolution. Annual Review of Phytopathology. 35:191–209.

Roossinck, M. J., and García-Arenal, F. 2015. Ecosystem simplification, biodiversity loss and plant virus emergence. Current Opinion in Virology. 10:56–62.

Roossinck, M. J., Martin, D. P., and Roumagnac, P. 2015. Plant Virus Metagenomics: Advances in Virus Discovery. Phytopathology™. 105:716–727.

Rubio, L., Galipienso, L., and Ferriol, I. 2020. Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. Frontiers in Plant Science. 11.

Rybicki, E. P. 2015. A Top Ten list for economically important plant viruses. Arch Virol. 160:17–20.

Salem, N., Mansour, A., Ciuffo, M., Falk, B. W., and Turina, M. 2016. A new tobamovirus infecting tomato crops in Jordan. Arch Virol. 161:503–506.

Schenk, M. F., Hamelink, R., van der Vlugt, R. A. A., Vermunt, A. M. W., Kaarsenmaker, R. C., and Stijger, I. C. C. M. M. 2010. The use of attenuated isolates of Pepino mosaic virus for cross-protection. Eur J Plant Pathol. 127:249–261.

Scholthof, K.-B. G., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., et al. 2011. Top 10 plant viruses in molecular plant pathology. Molecular Plant Pathology. 12:938–954.

Schreinemachers, P., Balasubramaniam, S., Boopathi, N. M., Ha, C. V., Kenyon, L., Praneetvatakul, S., et al. 2015. Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. Crop Protection. 75:115–123.

Sevik, M. A., and Arli-Sokmen, M. 2012. Estimation of the effect of Tomato spotted wilt virus (TSWV) infection on some yield components of tomato. Phytoparasitica. 40:87–93.

Simmonds, P., Aiewsakun, P., and Katzourakis, A. 2019. Prisoners of war — host adaptation and its constraints on virus evolution. Nat Rev Microbiol. 17:321–328.

Spence, N. J., Basham, J., Mumford, R. A., Hayman, G., Edmondson, R., and Jones, D. R. 2006. Effect of Pepino mosaic virus on the yield and quality of glasshouse-grown tomatoes in the UK. Plant Pathology. 55:595–606.

Syller, J. 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol Plant Pathol. 13:204–216.

Tack, A. J. M., and Dicke, M. 2013. Plant pathogens structure arthropod communities across multiple spatial and temporal scales. Functional Ecology. 27:633–645.

Tamburini, G., Bommarco, R., Wanger, T. C., Kremen, C., van der Heijden, M. G. A., Liebman, M., et al. 2020. Agricultural diversification promotes multiple ecosystem services without compromising yield. Science Advances. 6:eaba1715.

Tatineni, S., and Hein, G. L. 2023. Plant Viruses of Agricultural Importance: Current and Future Perspectives of Virus Disease Management Strategies. Phytopathology®. 113:117–141.

Thresh, J. M. 1982. Cropping Practices and Virus Spread. Annual Review of Phytopathology. 20:193–216.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., and Polasky, S. 2002. Agricultural sustainability and intensive production practices. Nature. 418:671–677.

Velasco, L., Ruiz, L., Galipienso, L., Rubio, L., and Janssen, D. 2020. A Historical Account of Viruses in Intensive Horticultural Crops in the Spanish Mediterranean Arc: New Challenges for a Sustainable Agriculture. Agronomy. 10:860.

Vermunt, A. M. W., and Kaarsemaker, R. C. 2017. Multi-genotype cross-protection against Pepino mosaic virus in tomato. Crop Protection. 96:116–122.

Villamor, D. E. V., Ho, T., Al Rwahnih, M., Martin, R. R., and Tzanetakis, I. E. 2019. High Throughput Sequencing For Plant Virus Detection and Discovery. Phytopathology®. 109:716–725.

Vunsh, R., Rosner, A., and Stein, A. 1990. The use of the polymerase chain reaction (PCR) for the detection of bean yellow mosaic virus in gladiolus. Annals of Applied Biology. 117:561–569.

Wezel, A., Casagrande, M., Celette, F., Vian, J.-F., Ferrer, A., and Peigné, J. 2014. Agroecological practices for sustainable agriculture. A review. Agronomy for Sustainable Development. 34:1–20.

Whitfield, A. E., Huot, O. B., Martin, K. M., Kondo, H., and Dietzgen, R. G. 2018. Plant rhabdoviruses—their origins and vector interactions. Current Opinion in Virology. 33:198–207.

Wilkinson, K., Grant, W. P., Green, L. E., Hunter, S., Jeger, M. J., Lowe, P., et al. 2011. Infectious diseases of animals and plants: an interdisciplinary approach. Philos Trans R Soc Lond B Biol Sci. 366:1933–1942.

Wren, J. D., Roossinck, M. J., Nelson, R. S., Scheets, K., Palmer, M. W., and Melcher, U. 2006. Plant Virus Biodiversity and Ecology. PLOS Biology. 4:e80.

Xu, C., Sun, X., Taylor, A., Jiao, C., Xu, Y., Cai, X., et al. 2017. Diversity, Distribution, and Evolution of Tomato Viruses in China Uncovered by Small RNA Sequencing. J. Virol. 91.

Yele, Y., and Poddar, N. 2020. Virus-insect Vector Interaction and their Management. In , p. 384–396.

Yuen, J. E., and Andersson, B. 2013. What is the evidence for sexual reproduction of Phytophthora infestans in Europe? Plant Pathology. 62:485–491.

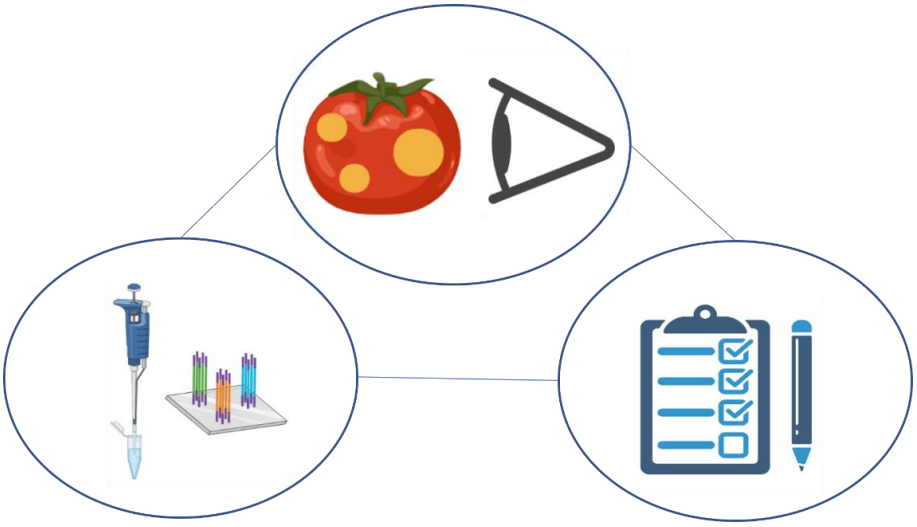
Zhang, S., Griffiths, J. S., Marchand, G., Bernards, M. A., and Wang, A. Tomato brown rugose fruit virus: An emerging and rapidly spreading plant RNA virus that threatens tomato production worldwide. Molecular Plant Pathology. n/a

Zhao, J., Zhang, X., Hong, Y., and Liu, Y. 2016. Chloroplast in Plant-Virus Interaction. Frontiers in Microbiology. 7.

Ziebell, H., and Carr, J. P. 2010. Cross-protection: a century of mystery. Adv Virus Res. 76:211–264.

Chapter 2

High Throughput Sequencing technologies complemented by grower's perception highlight the impact of tomato virome in diversified vegetable farms and a lack of awareness of emerging virus threats



Temple, C., Blouin, A. G., Tindale, S., Steyer, S., Marechal, K., Massart, S. (2023). High Throughput Sequencing technologies complemented by grower's perception highlight the impact of tomato virome in diversified vegetable farms and a lack of awareness of emerging virus threats. Front. Sustain. Food Syst. Sec. Crop Biology and Sustainability. Volume 7. doi: 10.3389/fsufs.2023.1139090.

Only text formatting was adapted to accommodate the published version.

Abstract

The number of small-scale diversified vegetable growers in industrialized countries has risen sharply over the last ten years. The risks associated with plant viruses in these systems have been barely studied in Europe, yet dramatic virus emergence events, such as tomato brown fruit rugose virus (ToBRFV), sometimes occur. We developed a methodology that aimed to understand better the implications related to viruses for tomato production in Belgian's vegetable farms by comparing growers' perception and the presence of plant-viral-like symptoms (visual inspection) with non-targeting detection of nearly all viruses present in the plants by high throughput sequencing technologies (HTS). Virus presence and impact were interpreted considering the farm's typology and cultural practices, and the grower's professional profiles. Overall, the data indicated that most growers have limited understanding of tomato viruses and are not concerned about them. Field observations were correlated to this perception as the prevalence of symptomatic plants was usually lower than 1%. However, important and potentially emergent viruses, mainly transmitted by insects, were detected in several farms. Notably, the presence of these viruses tended to be associated with the number of plant species grown per site (diversity) but not with a higher awareness of the growers regarding plant viral diseases, or a higher number of symptomatic plants. In addition, both HTS and perception analysis underlined the rising incidence and importance of an emergent virus: Physostegia chlorotic mottle virus. This study also revealed a notable lack of knowledge among producers regarding the highly contagious quarantine virus ToBRFV. Overall, the original methodology developed here, involving the integration of two separate fields of study (social science with phytopathology using HTS technologies), could be applied to other crops in other systems to identify emergent risks associated with plant viruses, and can highlight the communication needed with growers to mitigate epidemics. This exploratory investigation provides relevant insights which, ideally, would be further tested on wider samples to allow finer statistical treatment to be performed.

Keywords: Virome, grower's perception, high throughput sequencing, tomato, small-scale vegetable farms, Belgium

Introduction

Tomato (*Solanum lycopersicum L.*) is one of the most popular and valuable cultivated vegetables grown worldwide, with a gross production of 102.6 billion US dollars and yield estimated at 186.8 million tons (MT) in 2020 (FAOSTAT, 2020). Tomatoes are grown in diverse production systems (open fields, under plastic tunnels, hydroponics, high-tech greenhouses) for the fresh market or food industry. It is Europe's main produced vegetable, with 18 MT in 2021 (Eurostat, 2021). During 2021, the major part of the supply was in Italy (6.6 MT) and Spain (4.7 MT), where production occurs in open fields and tunnels, mainly for processing and export. In northern Europe, Poland (1.1 MT), the Nederlands (0.9MT), and Belgium (0.3MT) are the largest tomato producers, mainly for the production of fresh edible tomatoes in high-tech greenhouses (Eurostat, 2021).

Tomatoes are also grown by small-scale growers, and in gardens for local consumption (Benton Jones, 2007). In industrialized countries where small-scale growers almost disappeared during the green revolution, these production systems represent a recently expanding niche market (Morel and Leger, 2016, Laforge et al., 2018, Dumont et al., 2020). These small-scale growers promote human values and ecosystem welfare rather than profit maximization (Morel and Leger, 2016). Combining multiple logic and aspirations is indeed typical of agroecology-inspired growers (Plateau et al., 2021). Regarding farming practices, most of these growers aim to sustainably produce an extensive range of vegetables on soil, leaning on eco-system services, crop diversification, and rotations. Studies have shown that these systems have many advantages over conventional agriculture, especially for the environment and workers, as it reduces chemical and polluting inputs. In addition, these systems are supposed to have better resilience to climate change and plant diseases (King and Lively, 2012, Kremen et al., 2012, Mori et al., 2013). It has also been shown that multi-cropping and crop rotations increase yields in both organic and conventional cropping systems (Ponisio et al., 2015), encouraging the need for research on these agricultural practices to improve the productivity of sustainable agriculture methods.

In Belgium, there are two distinct sectors of tomato production. The most significant part of tomato production is dedicated to export and mass retailing. It is cultivated mainly in the northern part of the country (Flanders) by specialized tomato growers under high-tech greenhouses. A minor part of the production is achieved by small-scale growers producing tomatoes amongst other vegetables for local consumption. The number of these small-scale (< 2ha) growers has risen sharply over the last ten years in the southern part of Belgium, Wallonia (Dumont et al., 2020). A sociologic survey underlined that ethical and sociological factors were considered in growers' decision processes and that many growers are not from the agricultural sector (Dumont 2017). Most of these growers aimed to produce tomatoes on soil under tunnels or greenhouses and alternated tomatoes with other vegetables over a year (Dumont 2017).

Tomatoes are a sensitive crop: in all growing systems, the presence of pests and diseases (fungi, bacteria, viruses...), can jeopardize tomato crops, leading to important yield losses (Blancard, 2009). The characteristics of each pathosystem (the subset of an ecosystem in which the components include a host organism and an associated pathogen or parasite) determine specific strategies for plant pathogen control (Aranda, Freitas-Astúa, 2017). Thus, viral outbreaks are often related to unknown emerging diseases, for which diagnosis is the first step in disease management (Hanssen et al., 2010, Rubio et al., 2020). Viral diseases represent nearly half of the emerging plant diseases (Anderson et al., 2004) and tomato is the plant for which the most viruses are recorded (312 viral species in 2021, Rivarez et al., 2021). Plant viruses can be spread through insects, seeds, plant-to-plant contact, fungal spores, and other means.

Many environmental factors drive the emergence of plant viruses and their outbreaks by altering interactions between viruses, hosts, and vectors. For plant viruses, climate change and human activity, such as agriculture and trade, are the main factors influencing the outcome of these interactions (Jones et al., 2009, Elena et al., 2014). Elena et al., (2014) decoupled the emergence of new viruses into three phases. The first one requires a virus to jump from a host ("reservoir") to the same host in another ecological environment or to a new host ("spillover"). The second phase involves the adaptation of the virus in its new host/environment in which it develops the ability to be transmitted independently from the reservoir. The last phase is characterized by optimizing the virus transmission in this new host/environment and establishing the pathogen in the host population (Elena et al., 2014).

Although many viruses are known to infect tomatoes, all the interactions between the different actors of the pathosystem (vector, host, virus) must occur in a favorable environment in order for a virus to lead to an epidemic and subsequent consequences on the production. For annual crops, viruses can remain largely benign if their horizontal transmission is inefficient, resulting in low prevalence in the crop during a growing season. Therefore, the presence or absence of a virus in a given environment does not necessarily reflect the health of a field, and is not always equivalent to the disease impact ("viral disease risk"). Nevertheless, it is the first step in understanding and anticipating possible risks (MacDiarmid et al., 2013).

The development of high throughput sequencing (HTS) technologies significantly improved the detection of new and potentially emergent viruses in the last decade (Massart et al., 2014). For example, it helped to carry out surveillance studies for tomatoes without a priori knowledge of viruses (Xu et al., 2017, Villamor et al., 2019, Desbiez et al., 2020, Rivarez et al., 2021, Vučurović et al., 2021) This enabled the identification of emergent new viruses such as tomato brown rugose fruit virus (ToBRFV) (Salem et al., 2016), Physostegia chlorotic mottle virus (PhCMoV, Menzel et al., 2018) and facilitated the study of their evolution and epidemiology (Lefeuvre et al., 2019).

Of these emerging viral diseases, ToBRFV, which belongs to the *Tombamovirus* genus, has recently received the most attention from European scientists, policymakers and regulators and has sparked waves of regulatory action (Oladokun et al., 2019). ToBRFV is recommended to be regulated as a quarantine pest by EPPO

(https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list). This phenomenon is due to the association of ToBRFV with severe yield losses on tomato and pepper crops, combined with very high transmissibility (transmission by contact: tools, hands, clothes…) and stability it can remain active in the environment for months (Oladokun et al., 2019, Zhang et al., 2022).

PhCMoV also raised concerns as it is associated with extreme symptoms in tomato fruits. However, the low prevalence of the virus reported in the field so far makes it a lower threat. This virus has a vast host range spanning across nine families and infecting crops (eggplant, cucumber, crosne), weeds (galinsoga) and ornementals (hellebore, etc.) (Temple et al., 2021). It is likely transmitted by leafhoppers such as its close relative *Alphanuclorhabdovirus*, eggplant mottled dwarf virus (EMDV), and potato yellow dwarf virus (PYDV) (Babaie et al., 2003, Black, 1942).

Since viruses cannot be cured, their control mainly relies on i) their accurate detection and ii) the use of resistant varieties and/or limitation of their transmission, which can be either horizontal or/and vertical (seeds) and depends on the biological properties of each virus (Hull et al., 2014, Nicaise et al., 2014). Vega et al., (2019) propose to classify pathogens based on their dispersal and survival strategies, regardless of the taxonomic group to which they belong. This classification facilitates the interpretation of the occurrence of a viral disease in response to cultural practices.

The cost of plant testing may cause growers to rely mainly on their observations and knowledge to control virus infection in the field. In this context, it is crucial to determine virus perception by growers to understand the global virus-associated risks because their actions can affect the spread of viral diseases (Murray-Watson et al., 2022). In addition, growers are the first to observe the crops and to be conscious of their loss. Still, their perception of virus infection can sometimes be disconnected from reality, leading to inappropriate practices (Schreinemachers et al., 2015). Growers' perceptions and actions depend on several factors, including their knowledge of the disease, their virus-related experience and their production systems per se. Furthermore, the growers' actions are constrained by their financial means. In connection with the chosen production systems, growers' aspirations can also influence how they deal with viruses. Some producers may value growing vegetables more sustainably than maximizing their profit (Morel et al., 2016) and would tend to display different cultural practices than "conventional growers". These practices may play a role in virus presence and disease transmission. For example, growers who emphasize ecosystem welfare will be more reluctant to use insecticides because of their impact on non-targeted insects that might be important for other ecological functions (pollination, auxiliaires…). They may also be more likely to grow various tomato varieties, including old varieties that are not resistant to certain viruses such as tomato mosaic virus (Hansen et al., 2010) or, re-use their own seeds, which can promote the spread of seed-transmitted viruses.

Considering the importance of studying plant viruses (emergent or not) before they become a problem, the recent threat of ToBRFV in Belgium, and the context of climate change, and sustainable agricultural challenges, this study aims to evaluate

and compare the diversity of viruses in tomatoes grown on soil in diversified production systems with the associated risk perception of growers. Therefore, the first objective is to identify and understand the potential risks of viruses ("viral disease risk") in these production systems in Wallonia, Belgium.

Duong et al., (2019) emphasize that although biosecurity is the second largest risk mentioned by producers, there is a lack of research on socio-economic factors that explain risk perceptions, especially those that influence risk perceptions related to biosecurity. In addition, the role of cultural practices in virus presence and disease risk is critical to understand plant virus epidemiology (Jones, 2014), especially in sustainable agriculture where options for handling viral diseases are restricted. Therefore, the secondary objective is to interpret the results considering the farm typology and cultural practices, the grower's professional profiles, and the visual inspection of plant-viral-like symptoms, and to potentially evaluate what drives the presence of viruses and their impact. In this study, HTS will be used to assess the presence of viruses without a priori. Growers' perceptions will be compared to the presence of viruses and to the observations on the field to understand better the disease risk associated with these viruses within the different farms.

Material and methods

1.1. Study design

To better understand the implications of plant viruses for tomato production in Walloon vegetable farms, a three-tiered survey was designed: 1) interviews with the owner or manager of the tomato production, 2) field observations, and 3) analysis of the tomato virome through HTS technologies.

In 2020, a pilot survey was carried out with five growers and three members of the Interprofessional Center of vegetable growers (CIM, Regional extension services supervising vegetable production in Wallonia) to test and improve the study design to homogenize the questionnaire. Members of the CIM mentioned that they barely encountered outbreaks due to viral diseases in vegetable crops, including tomatoes: "most of the time, there are few virus-infected plants here and there, but viral epidemics are uncommon". For them, significant problems encountered during tomato culture are related to cryptogamic diseases.

A year after this pilot survey, the study was conducted with a standardized questionnaire with 21 tomato-growers in the province of Namur and Walloon Brabant at the end of the growing season (from August 18th to October 1st 2021) because the prevalence of viral diseases is usually highest at this time since the viral infection has been building up throughout the season.

1.2. Semi-structured interviews

Data collection

Growers' contact details were collected through the CIM, informal growers' network and by word of mouth.

WalOnMap (https://geoportail.wallonie.be/home.html) was used to determine the agricultural area where the different farms were located, and growers were grouped based on agricultural area and geographical relevance (growers located in the adjacent area of sandy-silty and silty were grouped and the ones in the adjacent Condroz and Famenne areas as well).

Interviews were conducted face to-face with the grower, informing the survey objective before the visit. During the exchanges, notes were taken, and interviews were audio recorded with the grower's approval. The questionnaire had two main objectives: 1) to describe the typology of the farms, grower's profiles, and cultural practices of tomato growing and, 2) to evaluate the perception of growers regarding tomato viral diseases. First, information about the farm (six variables: farm age, area, localization, number of vegetable species grown, organic label, number of employees (full-time equivalent)), tomato culture (six variables: number of plants and varieties grown, seedling origin, number of production years, disinfection of tool, usage of homemade seeds) and professional background of the interviewed person (four variables: registered at the CIM, number of years experience in the field, reconverted after another job, have relatives in the agricultural sector) were collected. The questionnaire is described in Supplementary data, and the answers for each farm are presented in Supplementary table 1. To obtain a global view of the answers, median, average, min and max values were calculated for the quantitative data and the sum for the binary data.

The second part of the questionnaire evaluated how growers perceived tomato viral diseases and which control measures were applied or envisioned. A mix of "open-ended" questions encouraging discussion and closed questions were asked in a specific order (Fig. 2-1).

At question Q5, pictures of viral symptoms induced by PhCMoV on different host plants were shown to assess if the growers recognized the symptoms (Supplementary Figure 1). Since viral symptoms are difficult to notice and sometimes resemble other stresses to the plant, respondents were able to validate or correct their answers to Q2, "In your opinion, have you ever experienced virus damage on tomatoes?"

PhCMoV symptoms were chosen because they are severe and can be easily identified in tomato fruits. They can also be mistaken with other important plant viruses known to be present in Belgium, such as ToBRFV or PepMV (Temple et al., 2021, Hanssen et al., 2009, EPPO Bulletin, 2020). In addition, it was the most frequently detected virus-causing symptom during the pilot survey in 2020.

In Q6, whether growers were aware of ToBRFV was investigated, as this virus was recently widely publicized by different stakeholders involved in the tomato production chain.

At the end of the interview, information on the biology of these two viruses (PhCMoV & ToBRFV), which require different control measures, were given to the growers.

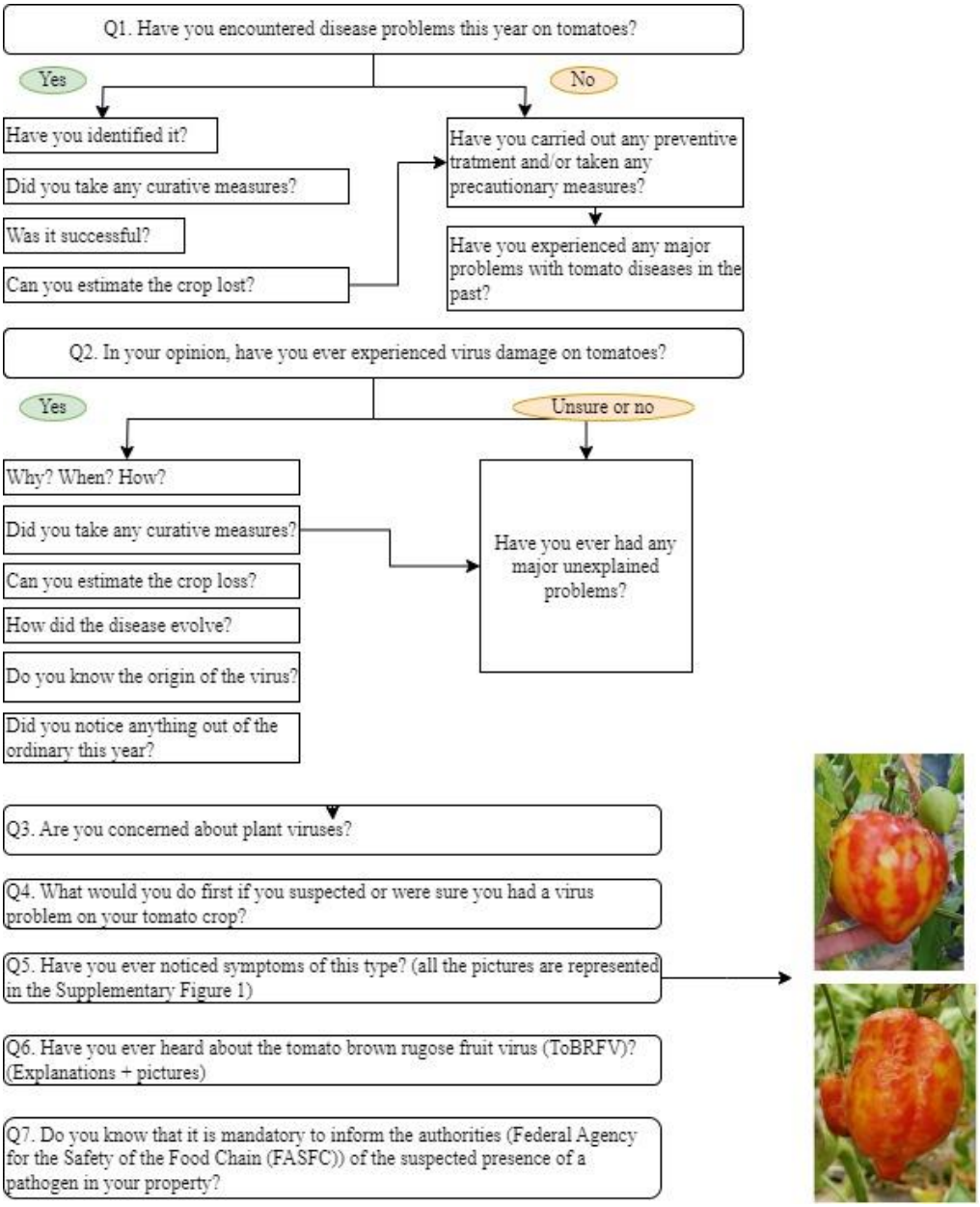


Figure 2-1. Questions related to virus growers' perception.

Data interpretation

The second part of the questionnaire investigated the perceptions and worries of the growers and allowed them to express themselves spontaneously. Interviews were first transcribed word by word, and answers were grouped based on the content of the replies. For instance, concerning Q2, responses were classified into four categories: 1. those who thought they already had viruses, 2. those who did not say "yes" clearly but who "did not rule out the possibility", 3. those who "did not know", and 4. those who "did not think" they already had viruses.

1.3. Observations and sampling

Prior to sample collection, each grower was explicitly told that if a quarantine virus (eg. ToBRFV) was detected, it was mandatory to notify Belgian's NPPO (Federal Agency for the Safety of Food chain). Thus oral consent to sample was sought from each grower.

In each farm, 100 asymptomatic tomato leaves were systemically collected following a W-shaped transect. When there were several tunnels on a farm, an equal number of plants was sampled per tunnel to reach 100 plants per farm. The tomato plants that showed viral-like symptoms (fruits: deformations, anomalies of coloration; leaves: vein clearing, deformation, mosaic; plant: reduced size) were pictured, counted, and collected in a separate bag.

Since the symptoms of PhCMoV can be easier to spot on eggplant than on tomato, eggplants were also examined when present on the farm. When the symptoms of PhCMoV were noticed on eggplants or tomatoes, the number of symptomatic plants was recorded, and at least three symptomatic plants per farm were collected.

In Belgium, 2021 was not optimal for outdoor tomato production due to very wet, cloudy, and cool weather (also exacerbated by storms and floods). These conditions favoured the development of fungal diseases, asphyxiated the root systems, and slowed down the ripening of the fruit. Consequently, some growers removed a part of the planting before the visit. These growers were listed (Supplementary Table 1).

1.4. Virus analysis

HTS

After the collection, 100 mg of fresh tomato asymptomatic leaves from the same farm (i.e. 10 g for 100 plants) were pooled in a filter bag and stored at -80°C. The symptomatic plants were also pooled per farm. The weight of material per plant varied according to the number of plants in total (5 g in total). After that, the samples were analyzed for viruses using a virion-associated nucleic acids enrichment protocol (VANA) before HTS on Illumina. The VANA protocol and library preparation used for the samples followed the method described by Maclot *et al.,* (2021) adapted from Filloux *et al.,* (2015).

In brief, 5 or 10 g of tissue was ground respectively in 25 or 50 mL of a cold Hanks' buffered salt solution (HBSS, composed of 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.07 g glucose, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 4.2 mM

$NaHCO_3$) using a tissue homogenizer. In a 50 mL falcon tube, the clarification was obtained from a centrifugation run of 10,000 g for 10 min at 4°C. Supernatant was then filtered through a 0.45 µm sterile syringe filter and 10,4 ml of supernatant was put into an ultracentrifuge tube (Beckman Ultra clear 14 mL tubes (#344085)). In each tube, 0.1 mL (1:100) of a clarified banana sample infected with banana bract mosaic virus (BBrMV) was added as an internal positive control to evaluate the analytical sensitivity of the test (Massart et al., 2022). Then, a sucrose cushion, made of 1 mL of 30% sucrose in 0.2 M potassium phosphate at pH 7.0, was deposited at the bottom of the tube. The extract was then centrifuged at 40 000 rpm for 2 hours at 4°C using the 50Ti rotor (Beckman). A protocol previously used in the laboratory and described in Maclot et al. (2021) was used for the library preparation.

PCR products were pooled by 6 to 12 according to the linkers and cleaned using the Nucleospin Gel and PCR cleanup (Macherey-Nagel). Samples containing asymptomatic plants were pooled separately from those containing symptomatic leaves to limit potential cross-contaminations of highly concentrated viruses in symptomatic pools. A positive external alien control containing infected beans with Endornavirus was processed simultaneously as the asymptomatic samples to monitor potential cross-contaminations as recommended in Massart et al., (2022).

Illumina library was prepared at GIGA Genomics (University of Liege, Belgium) using NEBNext Ultra II DNA library prep kit (New England BioLabs, US) and libraries were sequenced on the Illumina NextSeq500 sequencer for the generation 10 M of paired-end reads (2 x 150 base pair) per library. Resulting sequence reads were first demultiplexed according to the linker and trimmed from the adaptor, then quality trimming, pairing, and merging were performed using the Geneious R11 software platform (https://www.geneious.com) before de novo assembly with (RNA) SPAdes assembler 3.10.0 (Bankevich et al., 2012). Next, contigs were compared using tBlastx against a database of viruses and viroids sequences downloaded from NCBI in November 2021 (RefSeq virus database). Then, reads were mapped on the closest reference sequences using geneious parameters Medium-Low Sensitivity/ Fast. The presence of viruses was considered positive when the coverage (% of reference genome) was superior to 50% for most viruses and when the number of mapped reads on the closest genome reference was > 90. On another hand, the presence of tombusviruses and alphanecroviruses was assessed at a different threshold (12% of ref seq) since the number of reads which map on reference genomes, were very low compared to other viruses, such as the internal control BBrMV. This threshold was determined after a manual expertise assessment of the difference between contamination and low concentration of each virus (Rong et al., 2022).

RT-PCR for PhCMoV detection in eggplant

Eggplant leaves showing symptoms of PhCMoV were subjected to RNA extraction and RT-PCR for testing the presence of PhCMoV. RNA extraction followed the method described by Onate-Sanchez and Vicente-Carbajosa (2008). Then, the extracts were reverse transcribed using random hexamers and Tetro RT enzyme (Bioline). The obtained cDNA was amplified with the MangoTaq™ DNA Polymerase and the

primers described by Gaafar et al., (2018). Thermal cycling corresponded to: 94°C for 1 min, 35 cycles at 94°C for 15 s, 60°C for 20 s and 72°C for 45 s, with a final 72°C extension for 3 min. Amplicons were analyzed by electrophoresis on a 1% agarose gel in Tris-acetate-EDTA (TAE) buffer stained with GelRed® Nucleic Acid Gel Stain (Biotium) and visualized under UV light.

RT-PCR and sanger sequencing for confirmation of challenging HTS results

To confirm the detection of strawberry latent ringspot virus (SLRV) and carnation Italian ringspot virus (CIRV), RNA from the original 100 frozen leaves of the positive sample were re-extracted in pools of 25 with the Spectrum plant total RNA kit (Sigma-Aldrich) and tested by RT-PCR using the Titan One Tube RT-PCR kit. For the detection of SLRV, the primers SLRSV1/2 described by Bertolini et al., were used 2003 and for the detection of CIRV, primers were designed based on the consensus sequence using Geneious designing primer tool: CIRV- F: "CGTGGCAGTTACCAGACAGT", CIRV- R: "CTCCATCCCAACGTTCACCA" (product length: ~1kb). Amplicons were Sanger-sequenced, and the obtained sequences were searched against the NCBI database using BLASTn to confirm the presence of the virus.

The status of tomato mosaic virus (ToMV) was assessed in six samples where HTS yielded a small number of reads mapping the reference genome. The 100 frozen leaves for each of these sample and the positive sample were extracted in pools of 25 following the Spectrum plant total RNA kit (Sigma-Aldrich). RNA extracts were tested by RT-PCR using the Titan One Tube RT-PCR kit (Roche) and the primers of Li et al., 2018.

1.5. Data analyses

In this study, detected viruses were classified based on their transmission mode (insect-vector, soil, seeds, fungi, unknown, Table 2-1). Then, sites were grouped in categories based on the (transmission mode) class of viruses detected (Table 2-2, Supplementary table 1).

To evaluate the significance of the associations between the different variables, all the generated raw data in supplementary table 1 were tested two-by-two without a priori using Orange Mining's Sieve Plot diagrams widget (https://orangedatamining.com, Demsar et al., 2013). A Sieve Plot Diagram is a graphical method for visualizing the results of a chi-squared test of independence in a two-way contingency table. The chi-squared test is a statistical method that evaluates whether two categorical variables are independent of each other or whether they are related in some way. In the contingency table generated by this display, the area of each rectangle is proportional to the expected frequency, while the observed frequency is shown by the number of squares in each rectangle. The difference between observed and expected frequency (proportional to the standard Pearson residual) appears as the density of shading, using color to indicate whether the deviation from independence is positive (blue) or negative (red). The area of each rectangle is proportional to the expected frequency, while the observed frequency is shown by the number of squares

in each rectangle. Thereafter, using the relevance scoring options and attributes research of the Sieve Plot Diagram widget, the variables that were the most associated with interesting results were identified.

In addition, selected features related to the sites where viruses were transmitted through the most prevalent mode in symptomatic plants were compared to other sites. For that purpose, quantitative data were transformed into qualitative data based on the median value of each feature. In addition, all the data were normalized according to the number of growers with or without the viruses transmitted the same way.

Virus	Transmission	Family	Genus	Accronyme	Total nb of sites	Nb of sites where detections occurred in 21 AS plants pools	Nb of sites where detections occurred in 9 S plants pools
Potato virus Y	Insect	*Potyviridae*	*Potyvirus*	PVY	7	6	1
Cucumber mosaic virus	Insect	*Bromoviridae*	*Cucumovirus*	CMV	3	1	2
Physostegia chlorotic mottle virus	Insect	*Rhabdoviridae*	*Alphanucleorhabdovirus*	PhCMoV	6	0	6*
Southern tomato virus	Seeds	*Amalgaviridae*	*Amalgavirus*	STV	14	13	3
Tomato mosaic virus	Seeds, contact	*Virgaviridae*	*Tobamovirus*	ToMV	1	1	1
Moroccan pepper virus	Soil	*Tombusviridae*	*Tombusvirus*	MPV	2	1	2
Carnation Italian ringspot virus	Soil	*Tombusviridae*	*Tombusvirus*	CIRV	2	1	0
Tomato bunshy stunt virus	Soil	*Tombusviridae*	*Tombusvirus*	TBSV	1	0	1
Olive latent virus 1	Soil-borne fungi	*Tombusviridae*	*Alphanecrovirus*	OLV-1	1	1	0
Olive mild mosaic virus	Soil-borne fungi	*Tombusviridae*	*Alphanecrovirus*	OMM	1	1	0
Tobacco necrosis virus A	Soil-borne fungi	*Tombusviridae*	*Alphanecrovirus*	TNV-A	1	3	0
Strawberry latent ringspot virus	Nematode	*Secoviridae*	*Stralarivirus*	SLRV	1	1	0
Tomato matilda virus	Unknown	*Iflaviridae*	*Tomavirus*	TMaV	1	1	0

Table 2-1. Taxonomic characteristics of detected viruses and the number of sites where they were detected. Whether the detection occurred in symptomatic (S) or asymptomatic (AS) plants pools is indicated. *Three detections among the six were only made on symptomatic eggplant by RT-PCR (Supplementary table 2)

Transmission	Number of different viral species	Number of different viral families	Total number of positive sites detection	Nb of sites where detections occured in 21 AS plants pools	Nb of sites where detections occured in 9 S plants pools
Insects (PVY, CMV, PhCMoV)	3	3	13	7	7
Soil (MPV, CIRV, TBSV, OLV-1, OMM, TNV-A, SLRV)	7	2	9	9	4
Seeds / contact (ToMV, STV)	2	2	15	14	5
Unknown (TMaV)	2	1	1	1	0

Table 2-2. Number of sites where the different types of viruses were detected.

Results

1.6. General description of the farms, professional profile of the growers, and tomato culture

During the first step of the questionnaire, information was gathered on the general characteristics of the farm, the professional profile of the growers, and tomato cultivation practices. The detailed data are presented for each farm in Supplementary table 1.

The 21 surveyed growers sold their products locally (e.g. shop in the farm, markets, baskets, "pick your own"). The median time they had worked in vegetable farming was seven years, with only four years (median) of tomato production on the studied site (Table 2-3). This difference is because most of the growers worked on another vegetable farm before starting their own production. These growers stated that they had always grown tomatoes on their own farms. Less than half of the growers (9/21) had only worked in vegetable cultivation, while the others (12/21) switched careers after having worked in other sectors (Table 2-4). Only five respondents have relatives in the agricultural sector (Table 2-4).

Regarding growing systems, half of the respondents (11/21) grew vegetables under the organic label or were in the process of obtaining it. However, among the other half, most growers followed organic or agroecological farming practices without certification. Moreover, some of them explained that they don't need the organic label while following the practices because they are "close to their consumers" (notably in the case of pick-your-own farms). Therefore, it is challenging to mobilize this feature in the analyses.

Most of the surveyed growers produced many different vegetable species per year (median = 50) on a small surface area (median = 1.1 ha) (Table 2-3). The different crops (root vegetables, fruit vegetables, leafy green, cruciferous, marrow, aromatics…) were alternated on the same piece of land throughout the year. Only two growers stood out from the others by growing vegetables on a larger surface (INX-29: 4.5 ha and INX-37: 16 ha) (Supplementary table 1).

Regarding tomato production, the median number of tomato plants grown per year, per farm, was 1,000, with a median of 15 different cultivars. The growers bought their plants from nurseries or made seedlings from commercial or home-made seeds from the previous year (Table 2-3).

	F_Number of FTE	F_ Farm area (ha)	F_ Vegetables area (ha)	F_ Number of vegetables	G_Years of experience	T_ Production years	T_ Number of plants	T_Number of varieties
median	2.00	6.00	1.00	50.00	7.00	4.00	1000.00	15.00
average	3.39	22.50	2.01	44.90	7.60	5.00	1229.00	20.50
min	0.50	0.20	0.20	8.00	1.00	1.00	150.00	4.00
max	10.00	100.00	16.00	100.00	15.00	12.00	6500.00	80.00

Table 2-3. Description of the main quantitative characteristics of the farms (F); grower's profiles (G) and, tomato cultural practices (T) of the respondents (n=21). Median, Average, Min and Max values are indicated.

F_ Organic label	F_Geology	G_ members of the CIM	G_ Has always worked in agriculture	G_ Has relatives in the agricultural sector	T_ Seedlings origin	T_ Re-use of seeds	T_Disinfection of tools
Yes: 11 No: 10	S/S: 9 C/F: 12	Yes: 14 No: 7	Yes: 9 No: 12	Yes: 5 No: 17	Nursery: 9 Homemade: 9 Both: 3	Yes: 8* No: 13	Yes: 6 No: 15

Table 2-4. Description of the main qualitative characteristics related to the farms (F); grower's profiles (G) and, tomato cultural practices (T) of the respondents (n=21). S/S = silty and sandy-silty area, C/F = condroz and famenne area, CIM: Regional extension services supervising vegetable production in Wallonia, * none of them disinfected the seeds

1.7. General description of the grower perception, observations of viral-like symptoms and virus detected in the 21 farms

Grower perception

During the interviews, 20 out of 21 growers had faced cryptogamic diseases during the current year (Fig. 2-2 - Q1). One grower (INX-40) mentioned encountering problems with viruses (at the beginning of the interview process).

In the second question, specific to viral diseases, only four growers responded that they had faced virus infection, but it had never been problematic (except for the grower INX-40). Seven other growers did not rule out the possibility of having viruses, but were unsure. The rest of the growers "did not know" or "did not think" they ever had tomatoes infected by viruses (Fig. 2-2 - Q2). Interestingly, among the growers who did not think they had faced plant viruses or were unsure, no growers highlighted important unexplained troubles with tomato production at Q2. In addition, most growers (15/21) responded that they were not concerned about viruses (Fig. 2-2 - Q3).

Subsequently, the interviews revealed that many growers had little knowledge of viruses. Many (14/21) respondents naturally mentioned that they "didn't know about viruses", that they were "unaware of them", or that they "didn't know how to recognize them". The only grower that demonstrated his knowledge about plant viruses was the one with the most significant tomato production (INX-37). The lack of knowledge was exposed by question Q6 where less than half of the growers were aware of the potential danger posed by ToBRFV. They also mentioned that they did not know the list of quarantine pathogens or how to recognize them. In contrast, the growers seemed aware of fungal diseases as they all mentioned one disease name in Q1, and none highlighted their lack of knowledge about fungal diseases.

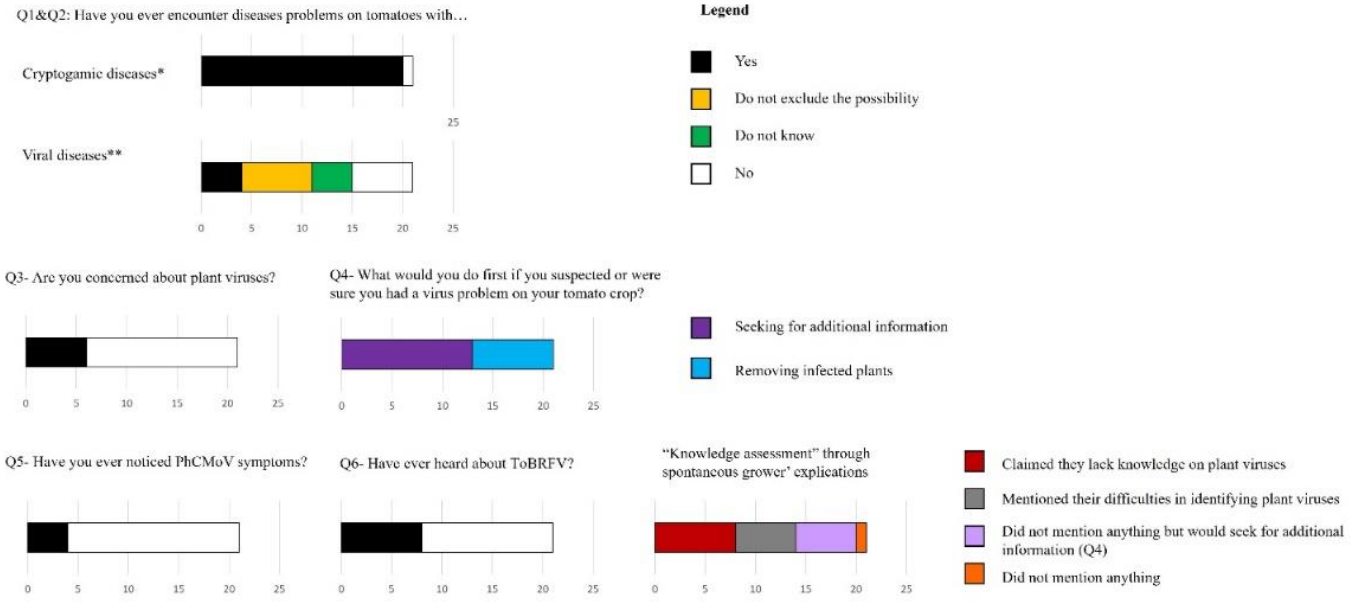


Figure 2-2. Perception of growers on viral diseases affecting tomato plants. * = during the current year, ** = since ever, n=21.

Observations of viral-like symptoms

In the parcels, 122 viral-like symptomatic tomato plants were collected in nine farms. In most cases, the proportion of symptomatic tomato plants was lower than 1% (Supplementary table 1).

Only one exception was noticed with the grower INX-40, who was aware of putatively virus-induced symptoms in his tomato crop (Q1, Q2). In this farm, 40/300 tomato plants (13%) showed strong viral symptoms like the ones associated with PhCMoV (Supplementary Fig. 3). In addition, one of the three tunnels from this farm was particularly impacted with 38/85 tomato plants showing these typical symptoms. In this tunnel, other plants were grown (cucumber, capsicum, mint, strawberry…), and the grower mentioned that he saw the symptoms related to PhCMoV in cucumber early in the season. In addition, three eggplants near the greenhouse also showed the symptoms of PhCMoV and tested positive.

In total, 30 eggplants showing PhCMoV symptoms (vein clearing on the leaves) were observed across five farms (including two farms where symptomatic tomatoes were observed, Supplementary table 2). The maximum number of symptomatic eggplants per farm was 11 (Supplementary table 2).

Virus detection

First, internal spike BBrMV was identified in all the asymptomatic pools with a number of reads mapped on the reference genome NC_009745 ranging between 5 and 99. Additionally, the external alien control (endornavirus) was detected in the two samples where it was expected with 27,690 and 43,744 reads mapped to the NC_038422 genome. There were no reads of endornavirus found in any other samples, indicating that the extent of cross-contamination was minimal after the filtering steps.

In total, 13 different viral species belonging to eight viral families were identified during this survey (Table 2-1). The number of virus species detected per farm varied between 1 and 4 (Supplementary Table 2).

These viruses were classified into four categories based on their transmission mode: transmitted by insects, by the soil (including virus transmitted by soil-borne fungi or nematodes), by seeds and/or contact, and the viruses for which the transmission is not known to date (Table 2-2). In total, three viral species transmitted by insects were detected across 16 different farms, seven viral species transmitted through the soil across nine farms, two viral species transmitted through seeds and/or contact across 14 farms, and one species for which biological data on its transmission was lacking, on one farm. In the symptomatic tomato plant pools, insect-borne viruses were the most prevalent, as they were detected across seven farms out of nine (Table 2-2).

The most frequently detected virus (n=14) was southern tomato virus (STV), a persistent virus transmitted by seeds. STV was detected more frequently in asymptomatic plant pools than in symptomatic pools (Table 2-1).

Then, the most prevalent viruses were the ones transmitted by insects: potato virus Y (PVY), PhCMoV and, cucumber mosaic virus (CMV) which were detected in respectively seven, six and three farms (Table 2-1).

HTS identified PhCMoV in three farms on symptomatic tomato showing fruit deformations and anomalies of colouration and by RT-PCR and on three additional farms on eggplants showing vein clearing on the leaves (Table 2-1). Overall, during the study, all the tested plants that showed PhCMoV symptoms were positive for this virus. PhCMoV was only detected in plants with typical PhCMoV symptoms and not in asymptomatic plants, whereas PVY was primarily identified in asymptomatic plants. Leafhoppers transmit PhCMoV, while aphids transmit PVY. These two viruses appeared on a farm where many symptomatic plants were found, including cucumber and eggplant and where the growers complained of tomato virus disease (INX-40). The symptoms observed were very characteristic of PhCMoV (Supplementary Figure 2c), which was detected by HTS on the pool of symptomatic tomatoes and by RT-PCR in four separate symptomatic tomato plants.

CMV was associated with symptoms in two symptomatic pools and in one asymptomatic pool (Supplementary Table 2a). Blancard, 2009).

Another virus transmitted by seeds, tomato mosaic virus (ToMV, Supplementary Figure 2b), was detected in one farm on asymptomatic and symptomatic plants. ToMV is a *Tobamovirus* which has been widely studied and is also transmitted by contact (Jones et al., 2014). RT-PCR confirmed the presence of the virus in the sample where the highest number of reads was found and not detected in all the other samples.

In addition, six different viral species belonging to the *Tombusvidae* family (tomato bushy stunt virus, moroccan pepper virus, olive latent virus 1, olive mild mosaic virus, tobacco necrosis virus A, CIRV) were primarily detected in asymptomatic plants (Table 2-1). These viruses are transmitted through the soil, mainly by soil-borne fungi and are not considered economically significant tomato pathogens (Yamamura et al., 2005).

Finally, SLRV (Secoviridae) and tomato matilda virus (TMaV; Ilflaviridae) were detected on asymptomatic plants only. SLRV is transmitted by a nematode, and the transmission mode of TMaV is unknown. This reports the first detection of SLRV and CIRV on tomatoes. RT-PCR and sanger sequencing was performed on the original plant samples and confirmed the presence of these viruses in tomatoes.

1.8. Associations

To investigate whether the presence of insect-borne viruses (PhCMoV, PVY, CMV) was associated with any specific metric related to the farms characteristics, cultural practices, grower's profiles or perception, the widget sieve diagram on orange mining was used on supplementary table 1, and associations with a p-value < 0.1 were noted.

Regarding cultural practices, the presence of these three viruses was associated with the increased number of different vegetable species grown per farm ("diversity"). Furthermore, these insect-borne viruses were also associated with the farms situated in the silty or sandy-silty area. Finally, regarding perception and actions, the presence

of PhCMoV, CMV and PVY was associated with the growers who would remove the virus-infected plants as a first reflex; with the ones who were not concerned about viruses; and with the ones who recognized PhCMoV symptoms (Fig. 2-3).

In a second step, selected features of interest related to the grower's perception, observations or other farms characteristics were converted into qualitative data if needed and evaluated against the presence of insect-borne viruses. This analysis showed that the farms with insect-borne viruses tended to have fewer tomato plants, which could be related to a higher number of different plant species per year, and with the growers who produce vegetables on smaller surfaces.

In addition, the four growers who thought they already had virus problems, and the one who recognized the symptoms of PhCMoV had insect-borne viruses (Fig. 2-4). Interestingly, most of these growers were not concerned about viruses (Fig. 2-4, Supplementary table 1). This perception can be related to their understanding that there was an absence of any significant problem of viruses on their farms, and highlights that the presence of PVY, CMV or PhCMoV is, in most cases, not associated with important yield losses. Also, three out of four growers who recognized the PhCMoV symptoms had PhCMoV in their farms. In addition, the growers who thought they had more than 30% losses during the current year due to fungal diseases on tomato were less likely than others to have insect-borne viruses, suggesting that yield losses from viral diseases, if any, were negligible in 2021 (Fig. 2-4).

Since there were no significant variations in how growers perceived viral diseases, it was difficult to compare the perception to the farms' characteristics and growers' profiles, and to understand if socio-economic factors can explain plant viruses risk perception. Only one grower claimed that he had problems with plant viruses. Using the widget sieve diagram on orange mining, it was tested whether being awared of ToBRFV (n=8) or being concerned by tomato viruses (n=6) was associated to any factors, and no significant associations were identified.

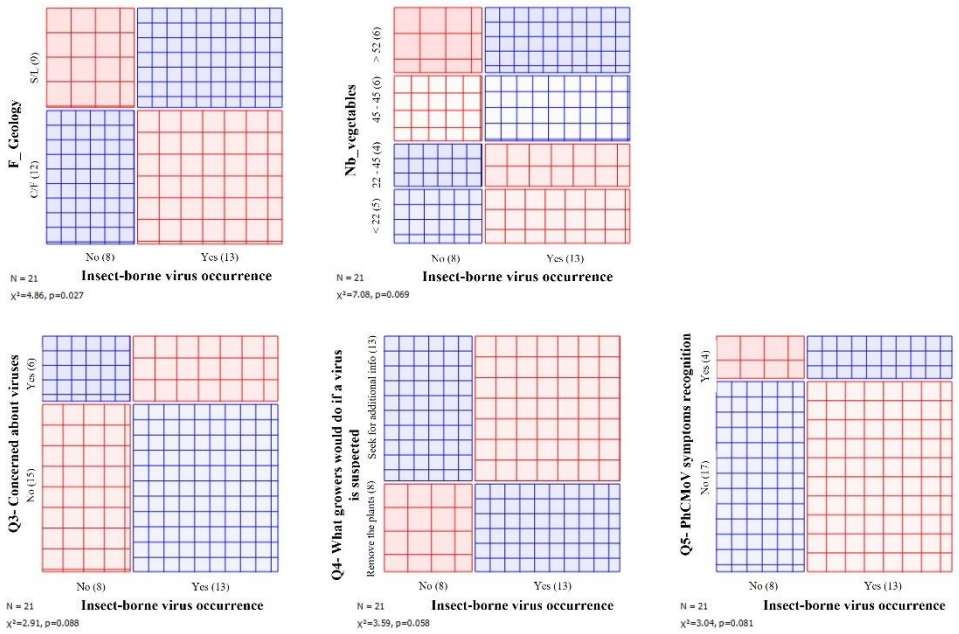


Figure 2-3. Associations between the presence of insect-borne viruses and different metrics. The area of each rectangle is proportional to the expected frequency, while the observed frequency is shown by the number of squares in each rectangle. The difference between observed and expected frequency (proportional to the standard Pearson residual) appears as the density of shading, using color to indicate whether the deviation from independence is positive (blue) or negative (red). S/S = silty and sandy-silty area, C/F = condroz and famenne area.

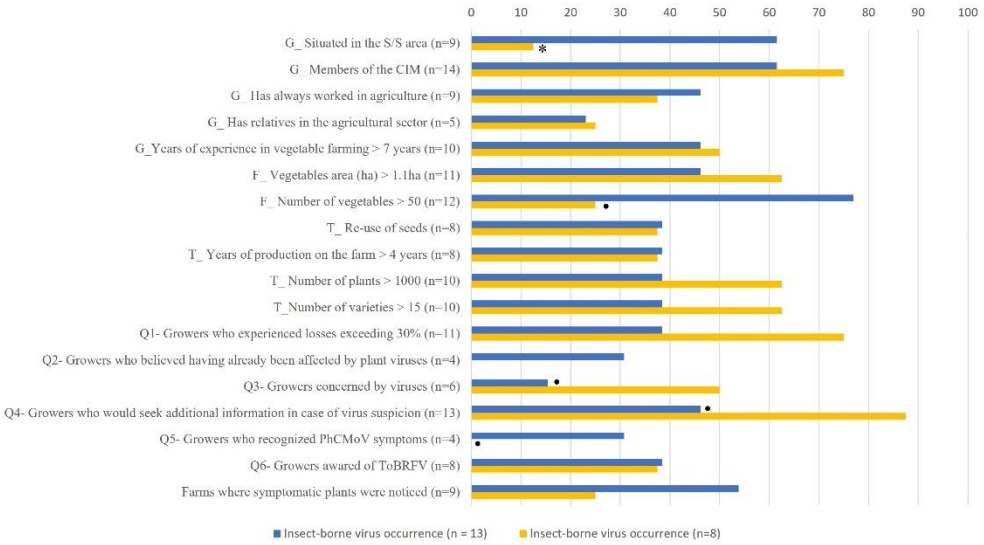


Figure 2-4. Percentage of growers with insect-borne viruses (blue) or not (yellow) vs selected characteristics related to the farms (F); grower's profiles (G) and, tomato cultural practices (T). S/S = silty and sandy-silty area, C/F = condroz and famenne area, CIM: Regional extension services supervising vegetable production in Wallonia, n represent the number of growers in each feature on which the analysis was based. The difference statistically highlighted with orange mining was indicated when $p < 0.1$ = . , $p < 0.05$ = *

Discussion

Overall, this study has provided knowledge of growers' perceptions regarding viruses, and the occurrence of tomato viruses, in small and diversified farms.

First, our selection process was representative because it was aligned with the classification of Dumont (2017): most of the interviewed vegetable growers produced a large diversity of vegetables sustainably on a surface area up to 2ha.

Second, their perception of viruses was somewhat unclear: most interviewed growers found it challenging to say whether they had problems linked to viral diseases. Hence, growers usually agreed that viruses were not a major problem in their tomato production. Observations of tomato plants in the field correlated with this perception, as a small number of symptomatic plants were found. However, some viruses known to affect tomato production were identified, such as ToMV, CMV and PhCMoV (Hanssen et al., 2010, Ullah et al., 2017, Mahjabeen et al., 2012, Temple et al., 2021).

Regarding the origin of the difference between perception, observations, and the presence of viruses, a first hypothesis (H1) is that the detected viruses do not cause significant problems in the agricultural system under study (low viral disease risk) and, perception and observations correlate well with reality. A second hypothesis (H2) might be that growers are unaware of the problem, and field observations are not

representative because symptoms of other diseases (fungal, bacterial, abiotic stress) might mask viral symptoms. This hypothesis can be supported by the fact that some growers admitted their lack of understanding of plant viruses or difficulties for identifying them.

While H2 cannot be entirely set aside, many elements suggest that H1 better explains the difference between perception and HTS results. Firstly, during the survey, all respondents admitted that they had a fungal disease problem and developed a control strategy for this issue. Many fungal disease problems in 2021 were observed due to wet weather conditions. The results related to the general tomato diseases perception suggested that growers seemed to be aware of the disease when they were severely impacted. Another reason supporting H1 is that the sole grower who reported a viral infection in their tomato crop experienced a significant virus outbreak. The observations and laboratory analysis were consistent with the growers' perception: many plants with typical PhCMoV symptoms were noticed, and the presence of the virus was confirmed. Finally, in the pilot survey, the regional extension services mentioned that viruses were not a significant problem in vegetable production, and that they rarely get requests on this subject. This information suggested that viral diseases were, at the time of study, not the most important diseases for tomato production in Wallonia, even if pathogenic viruses were detected. Many reasons can explain why a virus does not necessarily cause problems in a cropping system. For instance, its transmission from plant to plant might be inefficient resulting in a low number of plants infected, or the plants might get infected late, and the virus not have time to cause damage (Jeger, 2020, Trebicki, 2020).

Overall, these results contrast with the situation in the province of Alméria in Spain, where viruses represent a crucial threat to tomato production (Panno et al., 2019), and novel viruses are detected at a rate of 0.9 / year (Velasco et al., 2020). Consequently, the growers in Alméria recognize the significant risk of plant viruses (Velasco et al., 2020). It shows that growers' perception is also correlated to viral disease risk. Agricultural systems and tomato culture history may explain the difference. For example, in Alméria, tomatoes are grown intensively under plastic greenhouses at a very high density over 8,423 ha (in 2021) (Análisis de la campaña hortofrutícola. Campaña 2020/2021). There is almost no space between the parcels, which can facilitate the spread of viruses between fields. This is in contrast to the tomato growing systems observed in this study.

In the Nordic European countries, where most tomatoes are grown independently of seasonality in high-tech greenhouses, ToBRFV is of particular concern to intensive tomato growers because of the high risks associated with the movement of fruits, equipment and employees in greenhouses and warehouses (Zhang et al., 2022). This results in strict phytosanitary controls and a high level of awareness for all persons allowed to enter these greenhouses. In contrast, less than half of the small-scale producers interviewed in this study were aware of the ToBRFV despite the recent high-coverage for the disease in general, and specialized media. In addition, most of the small-scale growers were also unaware that reporting quarantine pests to authorities was mandatory. This highlights the existence of a gap in the information

channel between the plant health authorities and small-scale producers. Lack of awareness of harmful viruses may result from the system model in which most growers in this study operate: trade is often limited and local and other crops can compensate for low tomato yields in a given year. This can lead to a potential underlying infection that could remain unnoticed for some time. An outbreak would be very challenging to manage for a small farm, as a virus such as ToBRFV can survive in the environment over successive seasons. Whereas in a large high-tech glasshouse, a complete cleanup is costly and complex but possible. We found no socio-economic reasons or patterns to explain this low perception of ToBRFV. Nevertheless, this result demonstrates the value of addressing sociological factors that influence producers' decision-making in the face of a pathogen outbreak.

In this study, the most common viruses detected in symptomatic plants were insect-borne viruses. Subsequently, the characteristics that could possibly explain the presence of these viruses were investigated. The low number of producers (21) limited the scope of possible analyses. However, some patterns were detected, which might warrant further investigation. In terms of perception and action, the presence of insect-borne viruses was associated with growers who reported removing suspect plants; those who were not concerned about plant viruses; and those who recognized PhCMoV. The fact that the presence of PhCMoV is associated with growers who recognize its symptoms can be explained by the striking symptoms caused by this virus. Removing plants infected with viruses is often advisable to mitigate the spread of plant viruses. Therefore, it is surprising that growers of tomatoes infected with insect-borne viruses indicated that they would remove the symptomatic plant if they were sure of the viral origin of the symptoms. Furthermore, those who were not concerned about viruses (15/21) had the most insect-borne viruses. This result is also remarkable since insect-borne viruses were frequently detected in symptomatic plants. It suggests that growers did not notice the symptoms, and/or that these viruses were not associated with a high prevalence.

In addition, our analysis suggested that insect-borne viruses were more present in the farms where numerous different plant species were cultivated, and in the farms situated in the silty and sandy-silty areas. In both farms with and without insect-borne viruses, growers had the same profile: settled and working in agriculture for the same number of years. It is challenging to state whether being diversified (numerous plant species cultivated), located in the silty and sandy-silty area, or whether a range of characteristics explain the presence of these viruses, but the different elements can be justified.

Insect-borne viruses were detected more in the silty and sandy-silty area, where field crops such as cereals, sugar beet, potatoes, or flax are common. About half of the Walloon horticulture farms are in the silty area. It is recognized that the intensification of agriculture increased the emergence of viral diseases (Roossinck et al., 2015, Pinel-Galzi et al., 2015), and the proximity of large-scale cultivated plants next to the study plots could serve as virus reservoirs (Bernardo et al., 2018). In the Walloon context and our study case, potatoes have been primarily grown in the silty area for centuries.

This crop is the primary host of PVY; thus, the presence of potatoes might partly explain the presence of PVY in the farms situated in the silty region.

CMV, PhCMoV and PVY are insect-borne viruses with a broad host plant range which can affect tomato yield (Jones et al., 2014, Temple et al., 2021). The results tend to suggest that insect-borne viruses were more likely to be detected in the most diversified farms (which were not necessarily located in the silty/sandy silty area, data not shown). Growing a high number of vegetable plant species could increase the number of host plants harbouring insect vectors (Knops et al., 2002, Cook-Patton et al., 2011) or the number of host plants enabled to host viruses ("amplification effect", Keesing et al., 2006). Plant-cultivated diversity in small-scale production systems may also increase the first step of emergence: virus-host jump between wild and cultivated reservoirs as the number of potential new hosts is higher. In addition, these diversified farms have expanded in the past ten years throughout industrialized countries (including Belgium), and introduced new crops in the environment, a factor known to promote virus emergence (Elena et al., 2014). In this study, an emergent virus (PhCMoV) was detected in six farms out of 21. Even though the virus was detected in Germany since 2003 in a diversified system (Temple et al., 2021), its prevalence in Belgium could potentially be associated with the development of diversified farms.

Interestingly, in most cases, the presence of insect-borne viruses was not found to be associated with greater grower concern or an important number of symptomatic plants, suggesting that their presence did not pose a high risk for the production in diversified production systems. Although these results must be taken with precautions because of the relatively small number of studied farms, some studies postulate that plant-cultivated diversity protects against the spread of viral diseases (Haddad et al., 2009, Keesing et al., 2010, Pagán et al., 2012, Roossinck et al., 2015). Different protection mechanisms are involved, for example, creating a large genetic diversity for plants may lead to a dilution protective effect since pathogens have more chances of encountering resistant hosts in diverse habitats and more difficulties spreading (Liu et al., 2020; Keesing et al., 2021). In addition, plant diversity might increase the diversity of insect-vector but also of the predators, which should theoretically lead to an ecological balance (Cook-Patton et al., 2011, Haddad et al., 2010). It might also disturb the movement of insect vectors and thus reduce the spread of the disease (Power, 1991). Lichtenberg et al., 2017 demonstrate that organic farming and higher in-field plant diversity enhanced arthropod abundance, particularly for rare taxa, resulting in increased richness but decreased evenness. Our results tend to align with this statement since insect-borne viruses were more present in most diversified production systems but were not associated with higher viral disease risk. Nevertheless, more in-depth studies need to be undertaken to confirm this hypothesis. Alongside its other benefits, plant diversity has been shown to reduce the impact of other pathogens, such as fungi on crops and can be used for their management (Ratnasass et al., 2010, Mundt et al., 2022,). The agroecological paradigm states that pests and pathogens can be controlled, but not eliminated, through antagonistic ecological processes facilitated by crop genetic diversity or enhanced plant health (Van Bruggen et al., 2016). In addition, diversified farms increase the resilience of the

systems to biotic, abiotic and economic threats because if one crop is affected, growers can rely on other crops (Mori et al., 2013, Petersen-Rockney et al., 2021). These systems minimize the negative impact of agriculture on ecosystems and are less dependent on unstable geopolitical contexts, which can hamper food distribution from producers to consumers (Wezel et al., 2014, Hertel et al., 2021). Altogether, and in complement to the documented economic potential of diversified production systems (Van der Ploeg et al., 2019), these considerations suggest that additional research efforts on small-scale systems are needed to better optimize them.

Plant viruses constantly evolve, and monitoring them is essential to forecast emergence and outbreaks due to viral diseases. This study highlighted that some detected viruses would require more attention than others. For example, PhCMoV was widely distributed, associated with severe symptoms on few plants, and considered problematic by one grower. In addition, knowledge on the biology of this virus was limited (Temple et al., 2021). Thus, interactions of the virus with plants and vectors within the environment (reservoirs plant, transmission…) needs to be investigated more in-depth to understand how the disease can be developed, and to set up managment strategies.

Other not-well-known viruses were detected in tomatoes, among which two species (CIRV, SLRV) had not been detected in tomatoes before. However, since their presence was not associated with specific symptoms when no co-infection was noticed with other pathogenic viruses and growers did not notice it, their characterization might not be the priority for the moment. This study also reports, to our knowledge, the first detection of TMaV in Belgium. This virus was described for the first time in 2015 on tomato and did not seem to be associated with symptoms (Saqib et al., 2015). ToMV was detected on symptomatic plants showing typical ToMV symptoms (mosaic) and asymptomatic plants on a farm. ToMV was considered a severe threat to tomato production worldwide before the use of resistant cultivars and is currently re-emerging with the increased use of older, non-resistant cultivars (Hanssen et al., 2010). The virus is spread by seed and contact, and the grower affected with ToMV in this study was re-using their seeds from one year to another. Therefore, more information can be communicated to avoid practices that increase the likelihood of certain viruses spreading. Finally, STV was detected in 2/3rd of the farms (14/21) predominantly in asymptomatic plant pools, suggesting a potential minor role in plant pathogenicity.

Conclusion

The methodological approach used here makes it possible to obtain a holistic view of issues related to tomato viruses by combining, for the first time, a survey of growers perceptions with the characterization of the tomato virome by HTS technologies. In particular, the grower's perception enabled a more realistic understanding of the impact of the tomato virome on the field. Furthermore, it highlighted the need for better characterization of viruses detected by HTS, particularly when they can threaten production (e.g. PhCMoV). Overall, the results showed that, for this peculiar season

(2021), the presence of plant viruses was not necessarily linked to a high disease risk for the production according to the perception of growers and the symptom prevalence in diversified production systems in Belgium. Our preliminary investigation on what could explain these results suggests that more insect-borne viruses tended to be detected in the most diversified field. Since the detection were associated with low a viral disease pressure and a lack of concern from the growers, this finding supports the hypothesis that plant diversity might mitigate the impact of plant viruses on crops. Although these results need to be further validated over several years with a higher number of growers, they converge with the hypothesis of Keesing et al., (2010) which postulates that high biodiversity may provide a larger potential source of novel pathogens but would reduce further transmission for both long-established and newly-established emerging diseases. One of the findings of this study was that there was very little awareness of the virus threat among small-scale producers, as evidenced by the lack of knowledge of the ToBRFV, and the legal requirements associated with its presence. The individual interviews with the producers were an opportunity to inform them about known virus threats and their phytosanitary obligations.

This novel research approach, which combines the assessment of growers perception of the presence of viruses with their holistic laboratory detection, could be applied to other crops. The measured perception is valuable for directing technical communication towards knowledge gaps and for addressing phytosanitary risks. In this study, the detected viruses and their descriptions were provided to the interviewed growers and extension services. This is a starting point for raising farmers' awareness of viruses, which, combined with technical support on how to report viruses and control them safely, should lead to better preparedness and therefore mitigation of the impact of future viral disease outbreaks.

Supplementary materials

Access to the supplementary materials:
https://drive.google.com/drive/folders/1FYkCYs3Zr6s8AJK7rPHGoxdAliqWfL9F?usp=sharing

Funding

European Union´s Horizon 2020 Research and Innovation program under the Marie Sklodowska-Curie, Grant Agreement no. 813542.

Federal public service, public health, Belgium, Grant Agreement no. RT 18/3 SEVIPLANT 55.

Acknowledgments

The authors would like to thank the National Institute of Biology of Slovenia and especially Denis Kutnjak and Mark Paul Selda Rivarez for their help in bioinformatic analysis. We also acknowledge the Phytopathology team of ULiege Gembloux Agro-biotech (Gbx) Laurent Minet (Hortiforum asbl / Centre Technique Horticole de

Gembloux), and the members of the extension services (Centre Interprofessionel Maraicher) for their support and brainstorming effort in designing the survey and the questionnaire. Elisabeth Demonty and Pierre Hellin (Plant Virology Lab, CRA-W) are also thanks for their support in the laboratory analyzes. We are also very grateful to the growers who took the time to reply to the questions, allowed us to access their properties and collect samples. We thank Johan Rollin, Nuria Fontdevila, François Maclot (Gbx) and Sandrine Dury (CIRAD) for their support on analyzing the data and their helped in improving the manuscript. Finally, we thank Tomaž Curk for recommending the use of Orange data mining tool for our analyses.

Contribution to the field statement

Plant viruses can cause severe diseases in tomatoes, reducing yields and fruit quality. Once a virus has infected a plant, there is no cure. Therefore, viral diseases must be avoided prophylactically. The impact of viruses on tomatoes can vary depending on the biology of each virus, the cultivar and the environment. Diseases caused by viruses account for almost 50% of emerging plant diseases, reinforcing the need of awareness on these pathogens for growers. In highly industrialized countries, the number of small-scale vegetable growers relying on crop diversification and crop rotations has increased recently. However, the risks associated with viruses in these sustainable production systems are unknown. This study aimed to understand the viral disease risks threatening diversified production systems including tomatoes and the impact of cultural practices on these diseases. For this purpose, an innovative methodology was developed, combining for the first-time new technologies for virus identification (high throughput sequencing) and an questionnaire dedicated to the growers. The questionnaire aimed to understand growers' perception regarding viral diseases' impact and to describe the typology of the farms, which has been compared with the presence of viruses.

References

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., and Daszak, P. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology & Evolution. 19:535–544.

Aranda, M. A., and Freitas-Astúa, J. 2017. Ecology and diversity of plant viruses, and epidemiology of plant virus-induced diseases. Annals of Applied Biology. 171:1–4.

Babaie, G., and Izadpanah, K. 2003. Vector Transmission of Eggplant Mottled Dwarf Virus in Iran. Journal of Phytopathology. 151:679–682.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. 2012. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. J Comput Biol. 19:455–477.

Bernardo, P., Charles-Dominique, T., Barakat, M., Ortet, P., Fernandez, E., Filloux, D., et al. 2018. Geometagenomics illuminates the impact of agriculture on the distribution and prevalence of plant viruses at the ecosystem scale. ISME J. 12:173–184.

Bertolini, E., Olmos, A., López, M. M., and Cambra, M. 2003. Multiplex Nested Reverse Transcription-Polymerase Chain Reaction in a Single Tube for Sensitive and Simultaneous Detection of Four RNA Viruses and Pseudomonas savastanoi pv. savastanoi in Olive Trees. Phytopathology. 93:286–292.

Black, L. M. 1940. Strains of Potato Yellow-Dwarf Virus. American Journal of Botany. 27:386–392.

Blancard, D., Les maladies de la tomate - Identifier, connaître, maîtriser. Quae : des livres au coeur des sciences. Librairie Quae.

Cook-Patton, S. C., McArt, S. H., Parachnowitsch, A. L., Thaler, J. S., and Agrawal, A. A. 2011. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. Ecology. 92:915–923.

Desbiez, C., Wipf-Scheibel, C., Millot, P., Berthier, K., Girardot, G., Gognalons, P., et al. 2020. Distribution and evolution of the major viruses infecting cucurbitaceous and solanaceous crops in the French Mediterranean area. Virus Research. 286:198042.

Dumont, A. M., and Baret, P. 2017. *Classifications et cartographies des systèmes de production et commercialisation des légumes frais en Région wallonne (Belgique)*.

Dumont, A. M., Gasselin, P., and Baret, P. V. 2020. Transitions in agriculture: Three frameworks highlighting coexistence between a new agroecological configuration and an old, organic and conventional configuration of vegetable production in Wallonia (Belgium). Geoforum. 108:98–109.

Duong, T. T., Brewer, T., Luck, J., and Zander, K. 2019. A Global Review of Farmers' Perceptions of Agricultural Risks and Risk Management Strategies. Agriculture. 9:10.

Elena, S. F., Fraile, A., and García-Arenal, F. 2014. Chapter Three - Evolution and Emergence of Plant Viruses. In *Advances in Virus Research*, eds. Karl Maramorosch and Frederick A. Murphy. Academic Press, p. 161–191.

Filloux, D., Dallot, S., Delaunay, A., Galzi, S., Jacquot, E., and Roumagnac, P. 2015. Metagenomics Approaches Based on Virion-Associated Nucleic Acids (VANA): An Innovative Tool for Assessing Without A Priori Viral Diversity of Plants. Methods Mol Biol. 1302:249–257.

Fraile, A., and García-Arenal, F. 2016. Environment and evolution modulate plant virus pathogenesis. Current Opinion in Virology. 17:50–56.

Fukuhara, T., Tabara, M., Koiwa, H., and Takahashi, H. 2020. Effect of asymptomatic infection with southern tomato virus on tomato plants. Arch Virol. 165:11–20.

Gaafar, Y. Z. A., Abdelgalil, M. a. M., Knierim, D., Richert-Pöggeler, K. R., Menzel, W., Winter, S., et al. 2017. First Report of physostegia chlorotic mottle virus on Tomato (Solanum lycopersicum) in Germany. Plant Disease. 102:255–255.

Haddad, N. M., Crutsinger, G. M., Gross, K., Haarstad, J., Knops, J. M. H., and Tilman, D. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecology Letters. 12:1029–1039.

Hanssen, I. M., Lapidot, M., and Thomma, B. P. H. J. 2010. Emerging viral diseases of tomato crops. Mol. Plant Microbe Interact. 23:539–548.

Hertel, T., Elouafi, I., Tanticharoen, M., and Ewert, F. 2021. Diversification for enhanced food systems resilience. Nat Food. 2:832–834.

Jeger, M. J. 2020. The Epidemiology of Plant Virus Disease: Towards a New Synthesis. Plants (Basel). 9:1768.

Jones, R. a. c. 2014. Plant virus ecology and epidemiology: historical perspectives, recent progress and future prospects. Annals of Applied Biology. 164:320–347.

Jones, R. A. C. 2009. Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Research. 141:113–130.

Jr, B. 2007a. *Tomato Plant Culture: In the Field, Greenhouse, and Home Garden, Second Edition*.

Jr, J. B. J. 2007b. *Tomato Plant Culture: In the Field, Greenhouse, and Home Garden, Second Edition*. 2nd ed. Boca Raton: CRC Press.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., et al. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature. 468:647–652.

Keesing, F., Holt, R. D., and Ostfeld, R. S. 2006. Effects of species diversity on disease risk. Ecology Letters. 9:485–498.

Keesing, F., and Ostfeld, R. S. 2021. Dilution effects in disease ecology. Ecology Letters. 24:2490–2505.

King, K. C., and Lively, C. M. 2012. Does genetic diversity limit disease spread in natural host populations? Heredity. 109:199–203.

Knops, J. m. h., Tilman, D., Haddad, N. m., Naeem, S., Mitchell, C. e., Haarstad, J., et al. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecology Letters. 2:286–293.

Kremen, C., and Miles, A. 2012. Ecosystem Services in Biologically Diversified versus Conventional Farming Systems: Benefits, Externalities, and Trade-Offs. Ecology and Society. 17.

Laforge, J., Fenton, A., Lavalée-Picard, V., and McLachlan, S. 2018. New farmers and food policies in Canada. Canadian Food Studies / La Revue canadienne des études sur l'alimentation. 5:128–152.

Lefeuvre, P., Martin, D. P., Elena, S. F., Shepherd, D. N., Roumagnac, P., and Varsani, A. 2019. Evolution and ecology of plant viruses. Nature Reviews Microbiology. 17:632–644.

Li, Y., Tan, G., Lan, P., Zhang, A., Liu, Y., Li, R., et al. 2018. Detection of tobamoviruses by RT-PCR using a novel pair of degenerate primers. Journal of Virological Methods. 259:122–128.

Lichtenberg, E. M., Kennedy, C. M., Kremen, C., Batáry, P., Berendse, F., Bommarco, R., et al. 2017. A global synthesis of the effects of diversified farming systems on arthropod diversity within fields and across agricultural landscapes. Global Change Biology. 23:4946–4957.

Liu, X., Chen, L., Liu, M., García-Guzmán, G., Gilbert, G. S., and Zhou, S. 2020. Dilution effect of plant diversity on infectious diseases: latitudinal trend and biological context dependence. Oikos. 129:457–465.

MacDiarmid, R., Rodoni, B., Melcher, U., Ochoa-Corona, F., and Roossinck, M. 2013. Biosecurity Implications of New Technology and Discovery in Plant Virus Research. PLOS Pathogens. 9:e1003337.

Maclot, F. J., Debue, V., Blouin, A. G., Fontdevila Pareta, N., Tamisier, L., Filloux, D., et al. 2021. Identification, molecular and biological characterization of two novel secovirids in wild grass species in Belgium. Virus Research. 298:198397.

Mahjabeen, Akhtar, K. P., Sarwar, N., Saleem, M. Y., Asghar, M., Iqbal, Q., et al. 2012. Effect of cucumber mosaic virus infection on morphology, yield and phenolic contents of tomato. Archives of Phytopathology and Plant Protection. 45:766–782.

Massart, S., Adams, I., Al Rwahnih, M., Baeyen, S., Bilodeau, G. J., Blouin, A. G., et al. 2022. Guidelines for the reliable use of high throughput sequencing technologies to detect plant pathogens and pests. Peer Community Journal. 2.

Massart, S., Olmos, A., Jijakli, H., and Candresse, T. 2014. Current impact and future directions of high throughput sequencing in plant virus diagnostics. Virus Research. 188:90–96.

Menzel, W., Richert-Pöggeler, K., Winter, S., and Knierim, D. 2018. Characterization of a nucleorhabdovirus from Physostegia. Acta Horticulturae. 1193:29–38.

Morel, K., and Léger, F. 2016. A Conceptual Framework for Alternative Farmers' Strategic Choices: The Case of French Organic Market Gardening Microfarms. Agroecology and Sustainable Food Systems. 40.

Mori, A. S., Furukawa, T., and Sasaki, T. 2013. Response diversity determines the resilience of ecosystems to environmental change. Biological Reviews. 88:349–364.

Mundt, C. C. 2002. Use of Multiline Cultivars and Cultivar Mixtures for Disease Management. Annual Review of Phytopathology. 40:381–410.

Murray-Watson, R. E., Hamelin, F. M., and Cunniffe, N. J. 2022. How growers make decisions impacts plant disease control. PLOS Computational Biology. 18:e1010309.

Nicaise, V. 2014. Crop immunity against viruses: outcomes and future challenges. Frontiers in Plant Science. 5.

Oladokun, J. O., Halabi, M. H., Barua, P., and Nath, P. D. 2019. Tomato brown rugose fruit disease: current distribution, knowledge and future prospects. Plant Pathology. 68:1579–1586.

Oñate-Sánchez, L., and Vicente-Carbajosa, J. 2008. DNA-free RNA isolation protocols for Arabidopsis thaliana, including seeds and siliques. BMC Research Notes. 1:93.

Pagán, I., González-Jara, P., Moreno-Letelier, A., Rodelo-Urrego, M., Fraile, A., Piñero, D., et al. 2012. Effect of Biodiversity Changes in Disease Risk: Exploring Disease Emergence in a Plant-Virus System. PLoS Pathog. 8.

Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandić, A., et al. 2021. A Review of the Most Common and Economically Important Diseases That Undermine the Cultivation of Tomato Crop in the Mediterranean Basin. Agronomy. 11:2188.

Petersen-Rockney, M., Baur, P., Guzman, A., Bender, S. F., Calo, A., Castillo, F., et al. 2021. Narrow and Brittle or Broad and Nimble? Comparing Adaptive Capacity in Simplifying and Diversifying Farming Systems. Frontiers in Sustainable Food Systems. 5.

Pinel-Galzi, A., Traoré, O., Séré, Y., Hébrard, E., and Fargette, D. 2015. The biogeography of viral emergence: rice yellow mottle virus as a case study. Current Opinion in Virology. 10:7–13.

Plateau, L., Roudart, L., Hudon, M., and Maréchal, K. 2021. Opening the organisational black box to grasp the difficulties of agroecological transition. An empirical analysis of tensions in agroecological production cooperatives. Ecological Economics. 185:107048.

van der Ploeg, J. D., Barjolle, D., Bruil, J., Brunori, G., Costa Madureira, L. M., Dessein, J., et al. 2019. The economic potential of agroecology: Empirical evidence from Europe. Journal of Rural Studies. 71:46–61.

PM 7/146 (1) Tomato brown rugose fruit virus. 2021. EPPO Bulletin. 51:178–197.

Ponisio, L. C., M'Gonigle, L. K., Mace, K. C., Palomino, J., de Valpine, P., and Kremen, C. 2015. Diversification practices reduce organic to conventional yield gap. Proceedings of the Royal Society B: Biological Sciences. 282:20141396.

Power, A. G. 1991. Virus Spread and Vector Dynamics in Genetically Diverse Plant Populations. Ecology. 72:232–241.

Ratnadass, A., Fernandes, P., Avelino, J., and Habib, R. 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agron. Sustain. Dev. 32:273–303.

Rivarez, M. P. S., Vučurović, A., Mehle, N., Ravnikar, M., and Kutnjak, D. 2021. Global Advances in Tomato Virome Research: Current Status and the Impact of High-Throughput Sequencing. Front. Microbiol. 12.

Rong, W., Rollin, J., Hanafi, M., Roux, N., and Massart, S. 2022. Validation of high throughput sequencing as virus indexing test for Musa germplasm: performance criteria evaluation and contamination monitoring using an alien control. PhytoFrontiers™.

Roossinck, M. J., and García-Arenal, F. 2015. Ecosystem simplification, biodiversity loss and plant virus emergence. Current Opinion in Virology. 10:56–62.

Rubio, L., Galipienso, L., and Ferriol, I. 2020. Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. Front. Plant Sci. 11.

Salem, N., Mansour, A., Ciuffo, M., Falk, B. W., and Turina, M. 2016. A new tobamovirus infecting tomato crops in Jordan. Arch Virol. 161:503–506.

Saqib, M., Wylie, S. J., and Jones, M. G. K. 2015. Serendipitous identification of a new Iflavirus-like virus infecting tomato and its subsequent characterization. Plant Pathology. 64:519–527.

Schreinemachers, P., Balasubramaniam, S., Boopathi, N. M., Ha, C. V., Kenyon, L., Praneetvatakul, S., et al. 2015. Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. Crop Protection. 75:115–123.

Temple, C., Blouin, A. G., De Jonghe, K., Foucart, Y., Botermans, M., Westenberg, M., et al. 2022. Biological and Genetic Characterization of Physostegia Chlorotic Mottle Virus in Europe Based on Host Range, Location, and Time. Plant Disease. 106:2797–2807.

Tomato brown rugose fruit virus. 2020. EPPO Bulletin. 50:529–534.

Trebicki, P. 2020. Climate change and plant virus epidemiology. Virus Research. 286:198059.

Ullah, N., Ali, A., Ahmad, M., Fahim, M., Din, N., and Ahmad, F. 2017. Evaluation of tomato genotypes against Tomato mosaic virus (ToMV) and its effect on yield contributing parameters. Pakistan Journal of Botany. 49:1585–1592.

Vega, D., Gally, M. E., Romero, A. M., and Poggio, S. L. 2019. Functional groups of plant pathogens in agroecosystems: a review. Eur J Plant Pathol. 153:695–713.

Velasco, L., Ruiz, L., Galipienso, L., Rubio, L., and Janssen, D. 2020. A Historical Account of Viruses in Intensive Horticultural Crops in the Spanish Mediterranean Arc: New Challenges for a Sustainable Agriculture. Agronomy. 10:860.

Villamor, D. E. V., Ho, T., Al Rwahnih, M., Martin, R. R., and Tzanetakis, I. E. 2019. High Throughput Sequencing For Plant Virus Detection and Discovery. Phytopathology®. 109:716–725.

Vučurović, A., Kutnjak, D., Mehle, N., Stanković, I., Pecman, A., Bulajić, A., et al. 2021. Detection of Four New Tomato Viruses in Serbia Using Post Hoc High-Throughput

Sequencing Analysis of Samples From a Large-Scale Field Survey. Plant Disease. 105:2325–2332.

Wezel, A., Casagrande, M., Celette, F., Vian, J.-F., Ferrer, A., and Peigné, J. 2014. Agroecological practices for sustainable agriculture. A review. Agronomy for Sustainable Development. 34:1–20.

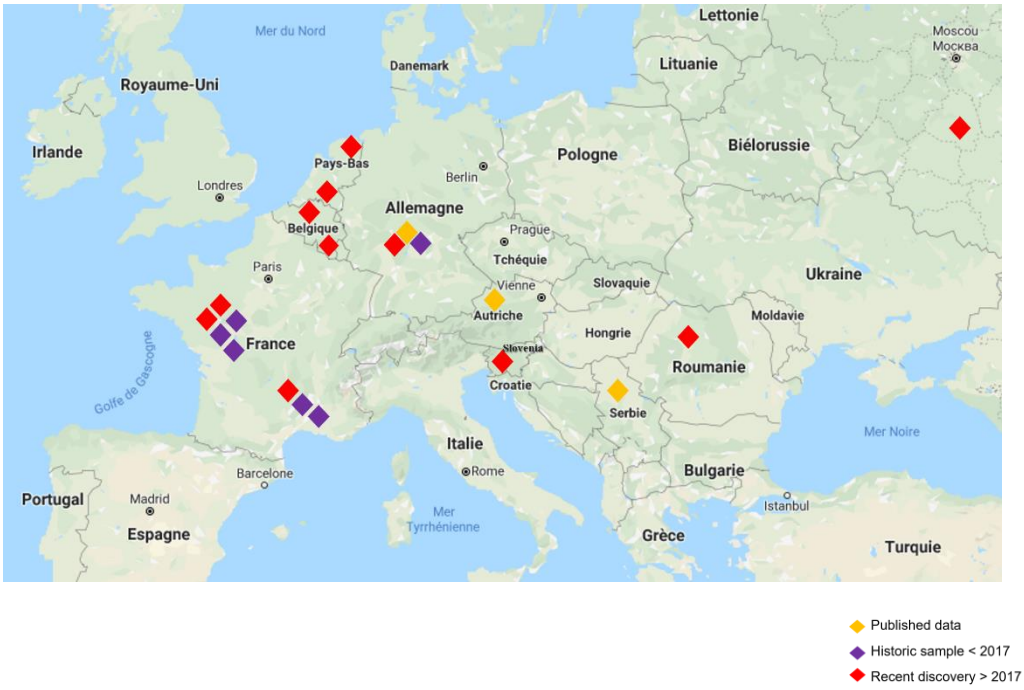
Xu, C., Sun, X., Taylor, A., Jiao, C., Xu, Y., Cai, X., et al. 2017. Diversity, Distribution, and Evolution of Tomato Viruses in China Uncovered by Small RNA Sequencing. J. Virol. 91.

Yamamura, Y., and Scholthof, H. B. 2005. Tomato bushy stunt virus: a resilient model system to study virus–plant interactions. Molecular Plant Pathology. 6:491–502.

Zhang, S., Griffiths, J. S., Marchand, G., Bernards, M. A., and Wang, A. Tomato brown rugose fruit virus: An emerging and rapidly spreading plant RNA virus that threatens tomato production worldwide. Molecular Plant Pathology. n/a

Chapter 3

Biological and Genetic Characterization of Physostegia Chlorotic Mottle Virus in Europe Based on Host Range, Location, and Time



Temple, C., Blouin, A. G., De Jonghe, K., Foucart, Y., Botermans, M., Westenberg, M., et al. (2022). Biological and Genetic Characterization of Physostegia Chlorotic Mottle Virus in Europe Based on Host Range, Location, and Time. *Plant Disease* 106, 2797–2807. doi: 10.1094/PDIS-12-21-2800-RE.

Only text formatting was adapted to accommodate the published version.

Abstract

Application of high-throughput sequencing (HTS) technologies enabled the first identification of Physostegia chlorotic mottle virus (PhCMoV) in 2018 in Austria. Subsequently, PhCMoV was detected in Germany and Serbia on tomatoes showing severe fruit mottling and ripening anomalies. We report here how pre-publication data-sharing resulted in an international collaboration across eight laboratories in five countries enabling an in-depth characterization of PhCMoV. The independent studies converged toward its recent identification in eight additional European countries and confirmed its presence in samples collected 20 ago (2002). The natural plant host range was expanded from two species to nine species across seven families, and we confirmed the association of PhCMoV presence with severe fruit symptoms on economically important crops such as tomato, eggplant, and cucumber. Mechanical inoculations of selected isolates in greenhouse established the causality of the symptoms on a new indexing host range. In addition, phylogenetic analysis showed a low genomic variation across the 29 near-complete genomes sequences available. Furthermore, a strong selection pressure within a specific ecosystem was suggested by nearly identical sequences recovered from different host plants through time. Overall, this study describes the European distribution of PhCMoV on multiple plant hosts, including economically important crops which the virus can cause severe fruit symptoms for. This work demonstrates how to efficiently improve knowledge on an emergent pathogen by sharing HTS data, and provides a solid knowledge foundation for further studies on plant rhabdoviruses.

Keywords: Emergent viruses, PhCMoV, datasharing, European distribution, high through put sequencing, mechanical inoculation, biological characterization

Introduction

High throughput sequencing (HTS) technologies have drastically increased the pace of new virus discoveries (Adams *et al.*, 2018). Following genome identification, biological characterization is essential to evaluate the scientific, commercial, and regulatory impact of plant pathogens (Massart *et al.*, 2017). Biological characterization of a new virus requires comprehensive knowledge on host range, vector, transmission, symptomatology, and general understanding of the epidemiology (Massart *et al.*, 2017). It requires studying of the virus to be done under controlled conditions, e.g., through mechanical inoculation or grafting (bioassays) (Roenhorst *et al.*, 2013). This is a long and complex process that does not follow the current pace of virus discoveries by HTS (Hou *et al.*, 2021). In this context, HTS data sharing across laboratories before publication can speed up the characterization of emerging viruses in plants, avoid duplication of effort and accelerate a more accurate pest risk analysis (Hammond et al., 2020). For example, it could describe the natural host range and symptoms associated with a new pathogen more extensively and identify crops that may have been impacted, or crops that could serve as reservoir. Merging HTS data from different sources (regions, countries) and data collected at different times (including historical samples) provides a better view of the spatial and temporal status and distribution of viruses, while improving knowledge on epidemiology from phylogenetic analyses. Additionally, historical samples and/or nucleic acids can be used to obtain valuable information on the viral origin, and gathering data from different sources about the conditions of discovery (host range, symptoms, etc.) can help to identify a possible route of invasion (Jones *et al.*, 2021).

Proving a causal relationship between a virus and a disease is one of the first steps in evaluating the risk associated with a new disease agent. However, complying with Koch's postulates is a time-consuming process that requires extensive bioassays (Fraile *et al.*, 2016; Adams *et al.*, 2018). To accelerate this characterization, Fox *et al.*, (2020) proposed a new approach based on epidemiological studies and statistical analysis that provide valuable insights into causal relationships. In that context, bringing together HTS data and bioassay results from various research laboratories offers a possibility to optimize the study of causal associations between a disease and a potential viral or virus-like agent.

Physostegia chlorotic mottle virus (PhCMoV) was first identified on *Physostegia virginiana* collected from Austria by Illumina HTS in 2014 (Menzel *et al.*, 2018). Subsequently, PhCMoV was detected in Germany and Serbia on tomatoes showing severe fruit marbling and ripening anomalies (Gaafar *et al.*, 2018; Vučurović *et al.*, 2021). PhCMoV has a negative-sense, single-stranded RNA (−ssRNA) genome of 13,321 nucleotides and belongs to the genus *Alphanucleorhabdovirus* of the family *Rhabdoviridae* (Kuhn *et al.*, 2020). Plant rhabdoviruses are believed to originate from insect viruses (Whitfield *et al.*, 2018; Dolja *et al.*, 2020); they are insect-vector-transmitted in a persistent and propagative manner (Jackson *et al.*, 2005). Seed or pollen transmission of plant rhabdoviruses has never been described (Jackson *et al.*, 2005).

Phylogenetic analyses of alphanucleorhabdoviruses revealed a close relationship between PhCMoV and eggplant mottled dwarf virus (EMDV), potato yellow dwarf virus (PYDV), constricta yellow dwarf virus (CYDV), and joa yellow blotch-associated virus (JYBaV) (Dietzgen *et al*., 2021; Bejerman *et al.*, 2021). Those five alphanucleorhabdoviruses share the same genome organization, which contains seven canonical open reading frames (ORFs) encoding (from 3' to 5') nucleoprotein (N), unknown function protein (X), phosphoprotein (P), putative movement protein (Y), matrix protein (M), glycoprotein (G) and large polymerase protein (L) (Dietzgen *et al*., 2021). These viruses infect dicotyledonous plants, and three of them (EMDV, PYDV and CYDV) are transmitted by leafhoppers. Vectors are still to be identified for the two most recently discovered viruses (JYBaV and PhCMoV). As genetically close plant rhabdoviruses are transmitted by a particular type of vector (Dietzgen *et al.,* 2021), PhCMoV and JYBaV are quite likely transmitted by a leafhopper, like how their close relatives alphanucleorhabdoviruses are.

Recent discoveries of PhCMoV in several European countries on various host plants – associated with severe symptoms in some cases - suggest that it is an emerging virus potentially harmful to economically important crops. Therefore, efficient and rapid characterization is required to establish proper risk assessment and manage the disease. In that context, eight European laboratories worked together to improve knowledge on PhCMoV biology, epidemiology, and genetic diversity.

Material and methods

The PhCMoV isolates that are reported here were independently detected and studied in different laboratories. PhCMoV was detected and identified from different plants during virus surveillance programs and plant pathogen diagnostic processes. For the detection, HTS and conventional sequencing (PCR and sanger sequencing) approaches were conducted. To confirm the presence of the virus after HTS detection, RT-PCR or mechanical transmission tests were performed. Ribo-depleted total RNA, double-stranded RNA (dsRNA) and Virion-Associated Nucleic Acids (VANA) were used as extraction and virus enrichment strategies prior to HTS on Illumina or Oxford Nanopore Technologies MinION platforms.

Host plant species, geographical location, date of collection, symptoms and sequencing method for each sample are indicated in Table 3-1. All the sequences were deposited in the GenBank database and the corresponding accession numbers are indicated in Table 3-1. The number of reads generated and horizontal coverage for each sample is indicated in the Supplementary Table 3-1. PhCMoV was detected from samples collected as part of surveys in Germany, Belgium, France, the Netherlands, and Slovenia and from symptomatic plants of different origins (the Netherlands, Russia, and Romania) submitted to the national reference laboratory in the Netherlands for diagnostics. The context of sample discovery is descripted for each sample in the following section, but the different sequencing methods and bioinformatic analyses are detailed in the Supplementary method 1.

Isolate name	Collection date	Origin: country (region or city)	Site (farm)	Original host [laboratory host if sequenced]	Symptoms on fruits	Symptoms on leaves [laboratory host if sequenced]	Detection method (D)/ confirmation (C) (protocol used)	Sequencing strategy (protocol used)	Coinfection with other viruses : Bioinformatic (B) or PCR results (PCR)	Bioinformatic pipeline (assemblers/ analyses)	Reference	Genbank accession
Fr_SM1	2002	France (Provence-Alpes-Côte d'Azur)	Site B	*Solanum melongena*	deformed	vein clearing, deformation	D: RT-PCR + sequencing (Alfaro-Fernandez et *al.,* 2009) /	Total RNA (a)	B: no	CLC workbench / Geneious	This study	MW934551
KY706238	2003	Germany, (State of Hess)	Site N	*Solanum lycopersicum*	unknown	unknown	C: RT-PCR (Gaafar *et al.,* 2018)	Total RNA + ribodepletion (Gaafar *et al.*, 2018)	B: no	Geneious	Gaafar *et al.*, 2018	KY706238
Fr_SL1	2011	France (Corse)	Site C	*Solanum lycopersicum*	deformed, uneven ripening, mottled	dwarf, mottled	D: RT-PCR + sequencing (Alfaro-Fernandez et *al.,* 2009) /	Total RNA (d)	B: Potato virus Y	Spades / Geneious	This study	MZ574100
232-12	2012	Serbia (Rasina District)	Site Q	*Solanum lycopersicum*	mottled, uneven ripening	mottled	RT-PCR (Vučurović *et al.*, 2021)	small RNA sequencing (Vucurovic *et al.,* 2021)	B: no	CLC workbench / Geneious (Vucurovic *et al.*, 2021)	Vučurović *et al.*, 2021	MT269810
238-12	2012	Serbia (Rasina District)	Site R	*Solanum lycopersicum*	mottled, uneven ripening	mottled	RT-PCR (Vučurović *et al.*, 2021)	small RNA sequencing (Vucurovic *et al.,* 2021)	B: no	CLC workbench / Geneious (Vucurovic *et al.*, 2021)	Vučurović *et al.*, 2021	MT269811
323-12	2012	Serbia (Jablanica District)	Site S	*Solanum lycopersicum*	mottled	ns	RT-PCR (Vučurović *et al.*, 2021)	small RNA sequencing (Vucurovic *et al.,* 2021)	B: Southern tomato virus	CLC workbench / Geneious (Vucurovic *et al.*, 2021)	Vučurović *et al.*, 2021	MT269812

Fr_SM2	2013	France, (Maine et Loire)	Site D	*Solanum melongena*	deformed, uneven ripening, mottled	vein clearing, plant: dwarf	D: RT-PCR + sequencing (Alfaro-Fernandez et *al.,* 2009) /	Total RNA (d)	B : no	Spades / Geneious	This study	MZ574102
Fr_SM3	2013	France, (Maine et Loire	Site D	*Solanum melongena*	deformed, uneven ripening, mottled	yellowing	D: RT-PCR + sequencing (Alfaro-Fernandez et *al.,* 2009) /	Total RNA (d)	B: no	Spades / Geneious	This study	MZ574103
Fr_SL2	2014	France (Provence-Alpes-Côte d'Azur)	Site E?	*Solanum lycopersicum*	deformed, uneven ripening, mottled	severe necrosis and dotted tasks (apical leaves)	D: RT-PCR + sequencing (Alfaro-Fernandez et *al.,* 2009) /	Total RNA (d)	B: Pepino mosaic virus + Squash mosaic virus	Spades / Geneious	This study	MZ574101
KX636164	2014	Austria	Site O	*Physostegia virginiana*	na	deformed, chlorosis and mottled	RT-PCR (Gaafar *et al.,* 2018)	Total RNA + ribodepletion (Gaafar *et al.*, 2018)	B: no	Geneious	Menzel *et al., 2018*	KX636164
KY859866	2015	Germany, (State of Hess)	Site N	*Solanum lycopersicum [N. benthamiana]*	marbling and discoloration	ns	C: RT-PCR (Gaafar *et al.,* 2018)	Total RNA (Gaafar *et al.*, 2017)	B: no	Geneious	Gaafar *et al.*, 2018	KY859866
Nd_SL1	2017	Netherlands	Site F	*Solanum lycopersicum [N.benthamiana]*	na	deformed, vein clearing	D: same as seq strategy C: mechanical inoculation	Total RNA (a)	B: no	CLC workbench / Geneious	This study	OK646027
Ru_SL1	2017	Russia	Site G	*Solanum lycopersicum [N.benthamiana]*	uneven ripening, mottled	mottled	D: same as seq strategy C: mechanical inoculation	Total RNA (a)	B: no	CLC workbench / Geneious	This study	OK646028

MK978541	2017	German y, (State of Hess)	Site N	*Solanum lycopersicum [N. benthamiana]*	marbling and discoloration	distortion and mild yellow spots	C: RT-PCR (Gaafar *et al.,* 2018)	dsRNA (Gaafar et al., 2020)	B: no	Geneious	Gaafar *et al.*, 2020	MK978541
MW848528	2017	German y, (State of Hess but different site)	Site P	*Solanum lycopersicum [N. benthamiana]*	marbling and discoloration	mild yellow spots	D: ELISA using JKI-2051	Total RNA + ribodepletion (Gaafar et al., 2020)	B: no	Geneious	Gaafar *et al., 2021*	MW848528
Nd_CS1	2018	Netherla nds (Zélande)	Site H	*Cucumis sativus*	pointed, deformed, vertical chlorotic stripes	interveinal chlorosis and sunken veins (rugosity)	D: same as seq strategy C: mechanical inoculation	Total RNA (a)	B: no	CLC workbench / Geneious	This study	OK646030
Nd_H1	2018	Netherla nds, (Gelderland)	Site I	*Helleborus*	na	vein clearing, chlorotic patterns and rings.	same as seq strategy	Total RNA (a)	B: no	CLC workbench / Geneious	This study	OK646029
Nd_H2	2018	Netherla nds (South Holland)	Site J	*Helleborus*	na	chlorosis next to veins and mosaic	D: same as seq strategy C: mechanical inoculation	Total RNA (a)	B: no	CLC workbench / Geneious	This study	OK646031
Fr_SM4	2018	France (Nouvelle Aquitaine)	Site K	*Solanum melongena*	deformed, uneven ripening, mottled	vein clearing, deformed	D: RT-PCR + sequencing (Alfaro-Fernandez et *al.,* 2009) /	Total RNA (d)	B: no	Spades / Geneious	This study	MZ574104
Be_SL1	2018	Belgium, (Gembloux)	Site A	*Solanum lycopersicum*	deformed, uneven ripening, mottled	vein clearing on apical leaves	C: RT-PCR (Gaafar *et al.,* 2018)	VANA (c)	B: no	Spades / Geneious	This study	MZ501244

Be_SM1	2019	Belgium, (Gembloux)	Site A	*Solanum melongena*	na	vein clearing	D: same as sequencing strategy / C: RT-PCR (Gaafar et al., 2018)	VANA (c)	B: no	Spades / Geneious	This study	MZ501245
Sl_SL1	2019	Slovenia	Site L	*Solanum lycopersicum*	deformed, uneven ripening, mottled	severe leaf curling and mottling P : dwarf	C: RT-PCR (Gaafar *et al.,* 2018)	Total RNA (f)	B and PCR: Tomato mosaic virus, Potato virus Y	CLC Genomics Workbench / SPAdes	This study	MW366749
Ro_SL1	2019	Romania	Site M	*Solanum lycopersicum [N.benthamiana]*	uneven ripening, mottled	na	D: same as seq strategy C: mechanical inoculation	Total RNA (a)	B: no	CLC workbench / Geneious	This study	OK646026
Be_IB1	2019	Belgium (Kruisem)	Site U	*Ipomoea batatas*	na	chlorosis, purple pattern	C: RT-PCR (own primers)	Total RNA (b)	B: Sweet potato feathery mottle virus	Own pipeline + VirusDetect + BWA/QUASR	This study	MZ389081
Be_SA1	2019	Belgium (Putte)	Site T	*Stachys affinis*	na	ns	C: RT-PCR (Gaafar *et al.,* 2018)	Total RNA (b)	B: no	Own pipeline + VirusDetect + BWA/QUASR	This study	MZ322957
Ge_CS1	2020	Germany, (State of Hess)	Site N	*Cucumis sativus*	na	mosaic, leaf curling, chlorotic spots and yellowing	C: RT-PCR (Gaafar *et al.,* 2018)	dsRNA e*	B: no	Minimap2 / Geneious	This study	MW081210
Be_GP1	2020	Belgium, (Gembloux)	Site A	*Galinsoga parviflora*	na	vein clearing	C: RT-PCR (Gaafar *et al.,* 2018)	Total RNA (d)	B: no	Spades / Geneious	This study	MZ574099
Be_PM1	2020	Belgium (Putte)	Site T	Persicaria maculosa	na	ns	C: RT-PCR (own primers)	Total RNA (b)	B: no	Own pipeline + VirusDetect + BWA/QUASR	This study	MZ389082

Be_IB2	2020	Belgium, import from Portugal	Site V	*Ipomoea batatas*	na	vein clearing , mosaic and stunting	C: RT-PCR (own primers)	Total RNA (b)	B: Sweet potato feathery mottle virus, Sweet potato chlorotic stunt virus, Potato virus Y	Own pipeline + VirusDetect + BWA/QUASR	This study	MW834321

Table 3-1. Sample references with collection year, localization (country and town if known), original host, symptoms, detection or confirmation method, sequencing strategy and bioinformatics pipeline used. NCBI GenBank accession numbers for each sequenced isolate and co-infection with other viruses are also presented.

Legend: a= protocol used by NVWA, b= protocol used by ILVO, c, d = protocol used by Uliege, e= protocol used by JKI, f= protocol used by NIB; All the samples were sequenced on Illumina plateform except for * = MinION ; na = non applicable (for example in the case that there is no fruit when the symptoms were recorded), ns = no symptoms observed

1.1. Samples origin and analysis by HTS

Samples Be_SL1, Be_SM1 and Be_GP1

During a survey on *Solanaceae* in 2019 in Belgium, one plant of *Solanum lycopersicum* (Be_SL1) was collected in a tomato production tunnel where multiple plants were showing deformed, mottled, and discolored fruits (Supplementary Figure 1). During this survey, the leaves of five plants of *Solanum melongena* (Be_SM1) showing strong vein clearing were collected in another tunnel and pooled together. The virus enrichment method VANA and the library preparation was performed on these two samples prior to HTS (Supplementary method 1) revealing the presence of PhCMoV.

A year later, multiple eggplant and tomato plants exhibited similar symptoms to those that were observed in 2019 within the same site we collected. Additionally, while inspecting *Capsicum annuum* grown in one of the tunnels, a plant of *Galinsonga parviflora* (Be_GP1) showing vein clearing was collected (Fig. 3-1h). RNA was extracted following the method described by Oñate-Sánchez *et al.,* (2008) and the detection of PhCMoV in these samples was confirmed by RT-PCR using the primers published by Gaafar *et al.,* (2018). The sample related to a new host (Be_GP1) was sequenced by Illumina after total RNA extraction, DNase treatment and ribodepletion (Supplementary method 1).

Sample Be_SA1, Be_IB1, Be_IB2 and Be_PM1

In the framework of a study on the phytosanitary risk of viruses in newly introduced crops in Belgium (PRONC, FPS project), eight samples of *Stacchys affinis* (crosne) and 91 samples of *Ipomoea batatas* (sweet potato) from imported vegetatively propagated starting material and seeds were collected in 2019 and 2020 in different production sites, including two community-supported agriculture (CSA) farms. The samples were taken randomly and not specifically based on the presence of symptoms. In a follow up survey, asymptomatic plants of several common weeds, including *Persicaria maculosa* (lady's thumb), *Chenopodium album* (lamb's quarters), *Solanum nigrum* (black nightshade), grasses (e.g., *Digitaria sanguinalis* (hairy crabgrass), *Echinochloa crus-galli* (cockspur grass)) and some other crops (*Physalis philadelphica* (tomatillo) and *Sechium edule* (chayote)), growing around the crosne plants were sampled. The samples were sequenced by Illumina after total RNA extraction, DNase treatment and ribodepletion (Supplementary method 1).

Sample Ge_CS1

During a survey in July 2020, nine cucumber samples (*Cucumis sativus* L.) showing mosaic leaf curling, chlorotic spots and yellowing symptoms were collected in an organic farm in Hesse State, Germany where the previously published PhCMoV isolates KY706238, MK948541 and KY859866 had been discovered (Gaafar *et al.,* 2018). Using immunosorbent electron microscopy (ISEM), cucumber mosaic virus was identified in five samples, while in one sample (Ge_CS1), bacilliform particles were observed suggesting the presence of a rhabdovirus. To identify the virus, double

stranded RNA (dsRNA) extraction followed by MinION sequencing were performed (Supplementary method 1).

Sample SL_SL1

In Slovenia, a survey of viruses in tomatoes and surrounding weeds was conducted in summer 2019. Thirty-five plant samples were collected within greenhouses at one farming site (10 tomato plants with symptoms resembling viral infection (which include, but not limited to, leaf curling, mosaic and yellowing leaves), 10 tomato plants without any visible disease symptoms and 15 samples from 12 wild species growing as weeds). The samples were sequenced by Illumina after total RNA extraction, DNase treatment and ribodepletion (Supplementary method 1).

Samples Nd_SL1, Ru_SL1, Nd_H1, Nd_H2, Ro_SL1 and Nd_CS1

From 2017-2019 symptomatic plant samples from the Netherlands, Russia and Romania were submitted to the NPPO of the Netherlands for diagnostic purposes. The samples were sequenced by Illumina after total RNA extraction, DNase treatment and ribodepletion (Supplementary method 1).

Sample Fr_SL1, Fr_SL2, Fr_SM2, Fr_SM3, Fr_SM4 and Fr_SM1

A survey conducted on cucurbits viruses in the south of France (Provence-Alpes Côte d'Azur) in summer 2008 revealed one cucumber sample with mosaic and yellowing leave symptoms (sample: 'C08-119'). ELISA performed with antisera produced for detecting the cucurbit-infecting viruses EMDV, zucchini yellow mosaic virus, watermelon mosaic virus, cucurbit aphid-borne yellows virus, cucumber mosaic virus, melon necrotic spot virus, moroccan watermelon mosaic virus, papaya ringspot virus and algerian watermelon mosaic virus only revealed the presence of EMDV (pers Eric Verdin).

In 2018, eggplant samples collected in Nouvelle-Aquitaine (Lot-et Garonne department) with vein clearing and deformed leave symptoms were simultaneously analysed in two french research institutes (ANSES and INRAE) by RT-PCR with primers published by Alfaro-Fernández *et al.*, (2011). Sanger sequencing was performed on amplicons of eggplant samples as well as cucumber samples collected in 2008. BLASTn homology search revealed the presence of PhCMoV for these two samples (Fr_SM4, 'C08-119').

From 2002 to 2018 in Southeastern France, several eggplant and tomato plants showing dwarfing, bumpy and marbling fruits and leaves, as well as deformations and vein clearing, were collected. Dip preparations were prepared from young symptomatic tomato or eggplant leaves, negatively stained with 1% phosphotungstic acid (PTA) and observed by transmission electron microscopy revealed the presence of characteristic bullets-shaped particles suggesting the presence of a rhabdovirus. Total RNA was extracted using the RNeasy Plant Mini kit® (Qiagen) according to the manufacturer's instructions and tested by RT-PCR with a set of primers designed for the detection of EMDV (Alfaro-Fernández *et al.*, 2011). The PCR products showed 78-81% nucleotide sequence identity with EMDV,

but since the PhCMoV sequence was not available at the time of detection (2002, 2011, 2013, 2014), the virus in the samples were categorized as "unknown nucleorhabdovirus" and set aside. Recently, these sequences were blasted to the updated NCBI database and the infection with PhCMoV were confirmed (96% to 98 of nucleotide sequence identity). Thereafter, the samples have been sequenced by HTS, Fr_SL1, Fr_SL2, Fr_SM2, Fr_SM3 and Fr_SM4 following the same methods described for Be_GP1 and Fr_SM1 following the same method described for Nd_SL1 (Supplementary method 1). Since 'C08-119' is the only sample that was not fully sequenced, the sequence of the amplicon generated with the primers of Alfaro-Fernández *et al.,* (2011) and obtained by Sanger sequencing is available in the Supplementary method 2 and on NCBI under the accession 'RYS_C08-119-A2021'.

1.2. Bioassays

Since mechanical transmission assays were performed in two distinct laboratories, JKI and NPPO-NL, the methods differ.

Sample isolate: KY882264 (JKI)

PhCMoV-infected *Nicotiana benthamiana* fresh leaves containing MW848528 isolate were used to inoculate *Chenopodiastrum murale, Chenopodium quinoa, Datura metel, D. stramonium, Hyoscyamus niger, Medicago sativa, N. benthamiana, N. occidentalis* 'hesperis', *N. occidentalis* 'P1', *N. tabacum* 'samsun', *Petroselinum crispum, Petunia* sp., *Physalis floridana*, *Solanum lycopersicum* 'harzfeuer', *S. lycopersicum* 'linda'. Four plants per species were inoculated. The method used for the inoculation was described before by Gaafar *et al.,* (2019). Briefly, symptomatic leaves were homogenized in Norit inoculation buffer (50mM phosphate buffer, pH 7, containing 1mM ethylenediaminetetraacetic acid (Na-EDTA), 20mM sodium diethyldithiocarbamic acid (Na-DIECA), 5mM thioglycolic acid, 0.75% activated charcoal and 30 mg Celite). Using a glass spatula, the homogenate was gently rubbed onto the leaves which were then rinsed with water. The inoculated plants were kept under greenhouse conditions (at 22 °C; photoperiod of 16 h light [natural daylight with additional growth light Phillips IP65, 400 W] and 8 h dark). Symptoms were observed four weeks post inoculation and the presence of PhCMoV was confirmed by RT-PCR with the primers of Gaafar *et al.,* (2018).

Sample isolate: Ru_SL1, Nd_SL1, Ro_SL1, Nd_CS1, Nd_H2 (NPPO-NL)

In the Netherlands, different PhCMoV isolates were tested on selected herbaceous indicators including *C. quinoa, D. stramonium, N. benthamiana, N. glutinosa, N. occidentalis P1, N. tabacum '*WB*', Physalis floridana, S. lycopericum*. Not all the plants were tested for all isolates, but the combinations are presented in Table 3-2. Three plants per species were inoculated. The method used for the inoculation protocol is described by Verhoeven & Roenhorst (2000). Briefly, 1g of infected frozen leaf material (*N. benthamiana* for Ru_SL1 and Nd_SL1 and original host for Ro_SL1, Nd_CS1 and Nd_H2) was ground in 10 mL inoculation buffer [0.02 M phosphate buffer pH 7.4, 2% (wlv) polyvinylpyrrolidone [(PVP; MW 10000)]. Plants were inoculated at a young stage (3-6 leaves) by gently rubbing the inoculum onto carborundum-dusted leaves. After inoculation, plants were rinsed with water and

placed in a glasshouse at 18-25°C with supplementary illumination for a day length of at least 14 h. Each isolate was inoculated to at least two plants per plant species and inspected visually for symptoms during the following seven weeks. The virus infection was confirmed by ELISA in all the inoculated plants (pers Marleen Botermans and Ruben Schoen).

	DSMZ- KX636164 (Menzel *et al.,* 2018)		JKI - KY859866 (Gaafar *et al.,* 2018) - HZ15-192		JKI - MW848528 (This study) - HZ16-558		NVWA - Ru_SL1 (This study)	NVWA - Nd_SL1 (This study)	NVWA - Ro_SL1 (This study)	NVWA - Nd_CS1 (This study)	NVWA - Nd_H2 (This study)
Inoculated test plant	**Symptoms**	**ELISA/ RT-PCR**	**Symptoms**	**ELISA/ RT-PCR**	**Symptoms**	**ELISA/ RT-PCR**	**Symptoms**	**Symptoms**	**Symptoms**	**Symptoms**	**Symptoms**
Chenopodium quinoa	-	-	y, m	+	-	-		-			
C. sativus	-	-									
Chenopodiastrum murale					-	-					
Datura stramonium	-	-			y	+	-	-			
D. metel	-	-			y	+					
Hyoscyamus niger					-	-					
Medicago sativa					-	-					
Nicotiana benthamiana	m	+	y, m	+	y, vc	+	m, r, g (5 wks p.i., 3/3)	m, r, g (5 wks p.i., 3/3)	m, r, g (4 wks p.i. (1/2))	vc, m, r, g (5 wks p.i. 2/3)	m, r, g (7 wks p.i., 3/3)
N. glutinosa	-	-			-	-		-			
N. occidentalis 'P1'	-	-	-	-	-	-	vc (4 wks p.i., 3/3)	vc, g (cl) (4 wks p.i., 3/3)	(0/2)	c (7 wks p.i., 1/3)	vc, g (7 wks p.i., 1/3)
N. tabacum samsunn					-	-					
N. tabacum 'WB'	vc	+					-	-			
N. clevelandii	m	+	y, m	+							
N. glutinosa '24A'	-	-			-	-					
N. hesperis	-	-			-	-					
N. occidentalis '37B'	vc	+	-	-							
Physalis floribunda							-	-			
Petroselinum crispum					-	-					
Petunia					-	-					
Physalis floridana	-	+			-	-					

Solanum lycopericum	-	-	-

Table 3-2. PhCMoV indexing host range study accross different laboratories (DSMZ, JKI and NVWA). Legend: c = chlorosis, cl = chlorotic lesions, g = growth reduction, ic = interveinal chlorosis, m = mottle, nl = necrotic lesions, r = rugosity, vc = vein clearing, y = yellowing, () = symptoms observed occasionally - = no symptoms, empty space = not tested, xx wks p.i. = number of weeks after inoculation before the observation of the first systemic (?) symptom, x/x = number of plants showing symptoms/ number of inoculated plants

1.3. Phylogenetic analyses

For the phylogenetic analyses, all the PhCMoV known sequences to date were used. This includes PhCMoV sequences published by Menzel *et al.*, (2018); Gaafar *et al.*, (2018); Gaafar *et al.*, 2021; Vučurović *et al.*, (2021) and the 21 new PhCMoV sequences generated in this study.

Prior to genome analysis, PhCMoV genomes were all trimmed to start at the sequence "CATGAGACT" (position 40 on genome KX636164) and end after "TGCACCTA" (position 13275 on genome KX636164). Phylogenetic analysis was carried out using the MEGA-X software (v10.1.8) (Kumar *et al.*, 2018). Sequence alignments were performed on near-complete genome using MUSCLE and the best DNA model was applied to the maximum-likelihood analysis (GTR+G+I model). Support for the branching patterns in the phylogenetic trees was determined by analyzing 1000 bootstrap replicates. For graphical representation, SIMPLOT software (version 3.5.1) was used to compare similarity of the genomic sequences of selected PhCMoV isolates to the reference query KX636164 (Window: 200bp, Step: 20bp, Gapstrim: On, Hamming). To improve the graphical representation, the analysis was limited to 16 PhCMoV isolates including the most divergent ones (Nd_SL1 and Nd_H2). KX636164 genome has been chosen as a reference because it is the first discovered PhCMoV isolate and longest genome (Menzel *et al.*, 2018).

Finally, to compare the genetic similarity between the different isolates for different genomic regions, the sequence of the N, X, P, Y, M, G and L ORF were extracted using Geneious software for all the isolates indicated in Table 3-1. Pairwise nucleotide and amino acid sequences identities were calculated for all isolates based on MUSCLE alignment (Muscle 3.8.425 by Robert C. Edgar).

Results

1.4. Natural host range and symptoms

In addition to the detection of PhCMoV in new host species belonging to the *Lamiaceae* (*Stachys affinis*) and *Solanaceae* (*Solanum melongena*) families, this study expands the natural host range of PhCMoV to seven new plant families: *Cucurbitaceae* (*Cucumis sativus*), *Ranunculaceae* (*Helleborus* sp.), *Convolvulaceae* (*Ipomoea batatas*), *Polygonaceae (Persicaria maculosa)* and *Asteraceae* (*Galinsoga parviflora*) (Table 3-1). These detections enabled the description of PhCMoV related symptoms on several hosts (Fig. 3-1, Table 3-1). Only samples with single infection by PhCMoV are shown in Fig. 3-1 (eggplant*:* Be_SM1, Fr_SM1, Fr_SM2, Fr_SM3, Fr_SM4, cucurbits: Nd_CS1, Ge_CS1; *Helleborus*: Nd_H1, Nd_H2; *G. parviflora*: Be_GP1; tomato: Nd_SL1, Ru_SL1, Ro_SL1, Be_SL1, Be_PM1).

As described previously by Gaafar *et al.*, (2018) and Vučurović *et al*., (2021) infected tomato fruit were unevenly ripened and mottled (Ru_SL1, Be_SL1, Ro_SL1) (Fig. 3-1a). In this study, some of the tomato infected fruit were also deformed

(Supplementary Figure 1). All PhCMoV infected tomato plants that bore mature fruit at the time of collection showed symptomatic fruit regardless of their growing conditions. The symptoms observed on tomato leaves were more variable: no symptom was observed on the leaves of Be_SL1 and Ro_SL1, mottled leaves were observed on Ru_SL1, and vein clearing and deformed leaves were observed on Nd_SL1 (Fig. 3-1b).

Like infected tomato, PhCMoV-infected eggplants showed deformed, unevenly ripened and mottled fruit (Fr_SM2, Fr_SM3, Fr_SM4) (Fig. 3-1c). Fr_SM1 showed deformed fruit. On the leaves, Be_SM1 and Fr_SM2 showed vein clearing (Fig. 3-1d), and Fr_SM3 showed, yellowing. Fr_SM4 and Fr_SM1 exhibited vein clearing and deformed leaves. Fr_SM2 showed dwarfism. Sample Be_SM1 grouped five eggplants, all of which showed vein clearing in new leaves. No mixed infection occurred in this bulk sample, which strongly suggests that PhCMoV was the causal agent of the symptoms observed on all the plants. No fruit was present at the time of sampling.

Infected cucumber fruit were pointed, deformed, and showed vertical chlorotic stripes (Nd_CS1) (Fig. 3-1e). The leaves exhibited interveinal chlorosis and sunken veins (Supplementary Figure S1), leaf curling, chlorotic spots, and yellowing symptoms (Fig. 3-1f). Finally, *G. parviflora* (Be_GP1) and *Helleborus* sp. (Nd_H1, Nd_H2) leaves showed vein clearing (Fig. 3-1 g, h). No symptom was observed on *Stachys affinis* or *Persicaria maculosa* at the time of collection.

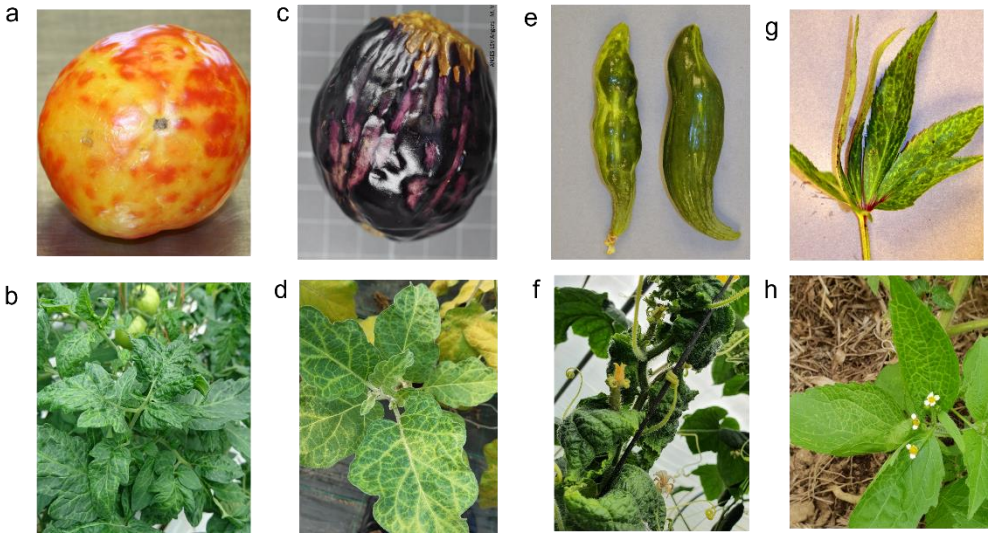


Fig. 3-1. Pictures of natural Physostegia chlorotic mottle virus (PhCMoV)-infected plants. A and B, symptoms of PhCMoV on infected Solanum lycopersicum fruits (Ro_SL1) and leaves (Nd_SL1); C and D, Solanum melongena fruit (Fr_SM4) and leaves (Be_SM1); E and F, Cucumis sativus fruits (Nd_CS1) and leaves (Ge_CS1); and G and H, Helleborus leaves (Nd_H1) Galinsoga parviflora (Be_GP1). No coinfections with other viruses occurred in these samples.

1.5. Experimental host range and symptoms

We conducted independent experiments to investigate the indexing host range of PhCMoV. The results of Menzel *et al*., (2018) (isolate KX636164) and Gafaar *et al*., (2018) (isolate KY859866) were grouped with our own present results to have a more complete overview (Table 3-2).

At JKI, PhCMoV (MW848528) was mechanically transmitted to *D. stramonium, D. metel* and *N. benthamiana* and induced yellowing and vein clearing four weeks after inoculation. Inoculation of the other 13 plant species tested failed (Table 3-2). This result differs from previous published reports, where *C. quinoa* and *P. floribunda* were successfully inoculated whereas inoculation of *D. stramonium* and *D. metel* failed.

In The Netherlands, five PhCMoV isolates where single infection occurred (Nd_SL1, Nd_CS1, Nd_H2, Ru_SL1, Ro_SL1) were mechanically transmitted to different indicator plants (*D. stramonium, N. benthamiana, N. occidentalis P1, N. tabacum 'WB', P. floribunda, S. lycopersicum*). An overview is presented in Table 3-2.

In all experiments, *N. benthamiana* displayed systemic symptoms four to seven weeks post inoculation (Table 3-2) and Nd_SL1, Nd_CS1, Nd_H2, Ru_SL1 induced systemic symptoms in *N. occidentalis P1* four to seven weeks post inoculation.

1.6. Extended distribution across Europe since 2002

This study provides an overview of the wide European geographical distribution of PhCMoV: its presence is confirmed in six additional countries besides Germany, Austria, and Serbia where the virus was previously reported (Menzel *et al.,* 2018; Gaafar *et al.*, 2018; Vučurović *et al.,* 2021): Russia, Romania, Slovenia, The Netherlands, Belgium, and France (Table 3-1).

Although most of the detections are recent, re-analysis of historic *S. melongena* samples (Fr_SM1) showed that PhCMoV was present in France as early as 2002. A cucumber sample collected in France in 2008 and originally diagnosed as EMDV by ELISA using in-house antiserum was re-analysed and diagnosed as PhCMoV by RT-PCR. This shows that some EMDV antiserums used by ELISA can cross-react with PhCMoV and lead to incorrect diagnosis.

1.7. Phylogenetic analysis of the genomes

In total, 21 new near-complete PhCMoV sequences were generated during this study, and their evolutionary relationships were investigated alongside all PhCMoV, EMDV and PYDV complete genomes available from the GenBank database on a maximum-likelihood (ML) tree (Supplementary Figure 2). Supported by bootstrap values of 1000, the analysis did not show any clustering according to host plant, country of origin or year of collection (Fig. 3-2). However, isolates collected from the same site (same farm) A, B, N or T grouped together regardless of the collection date or host plant (Fig. 3-2). This was particularly obvious for some of the samples from Germany, namely Ge_CS1, KY706238, KY859866, MK978541, and MW848528. They were collected at the same site (Hesse state) and grouped together despite their

collection date (from 2003 to 2020) and host plants (cucumber, tomato). Be_SL1, Be_GP1 and Be_SM1 were also collected on the same farm (Gembloux, Belgium) one year apart on three distinct host plants, but have almost identical genome sequences (100% nt id; Supplementary Figure 3). Similarly, Fr_SM2 and Fr_SM3 were collected at the same location and clustered together (Fig. 3-2). Interestingly, Be_SA1 and Be_PM1 sampled from the same farm also clustered together, along with Nd_CS1 which was isolated from a different country and host family (Fig. 3-2).Overall, all the sequences from samples collected on a same site clustered together, but the clusters did not all represent a geographical point.

To better understand the evolutionary relationships among PhCMoV isolates, nucleotides and amino acid identities were calculated from the alignment of nearly complete genome sequences and for each ORF (Fig. 3-3b). Relatively low genetic variability was observed for the near-complete genomic sequences (>93% nt id) in 28 isolates out of 29 (Fig. 3-3b). Nd_SL1 isolate was the most divergent isolate with 81-82% of nucleotide sequence identity (nts id) compared to the other 28 genomes (Fig. 3-3b). However, when the amino acid sequence identities (aa id) of the different isolates were compared, the variability of Nd_SL1 ranged among the average pairwise identities of the other isolates for most ORFs (N, P, Y, M, G) (Fig. 3-3b). Using Simplot to observe the sequence similarity along the genome, a clear drop was visible in the intergenic regions located in-between the coding regions (Fig. 3-3a). Overall, for all isolates except Nd_SL1, the ORF encoding protein L was the most conserved gene, with a percentage of aa id > 99%. It was followed by the ORF encoding protein G (aa id > 97%), and by those encoding proteins M, Y and P (aa id > 96%), N (aa id > 95%) and X (aa id > 88 %).

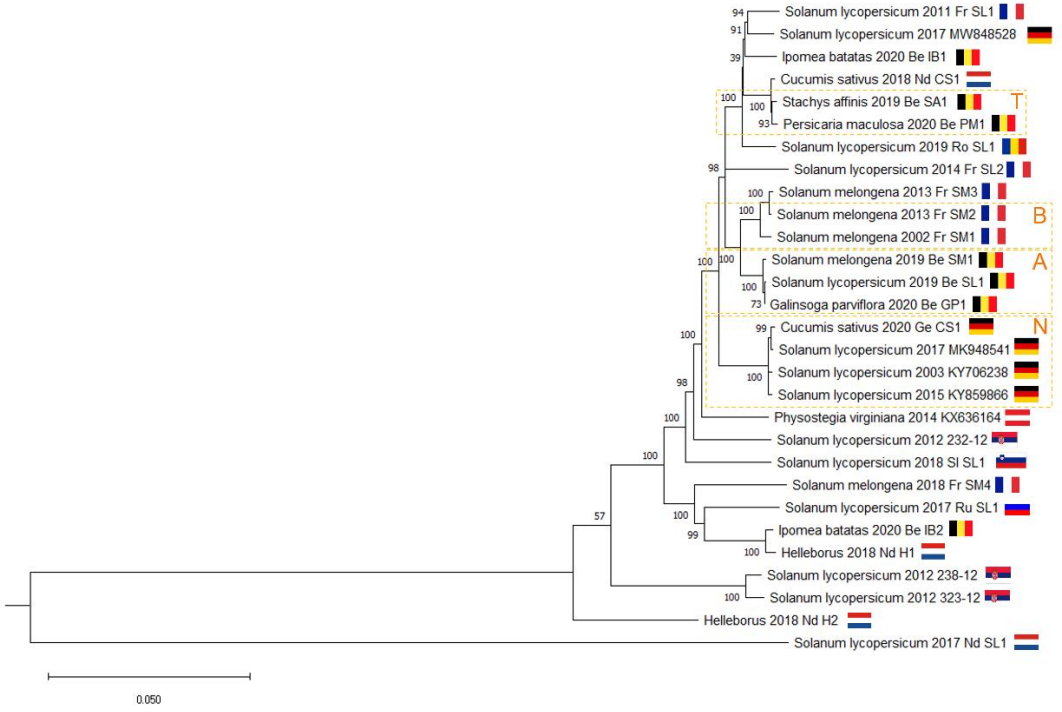
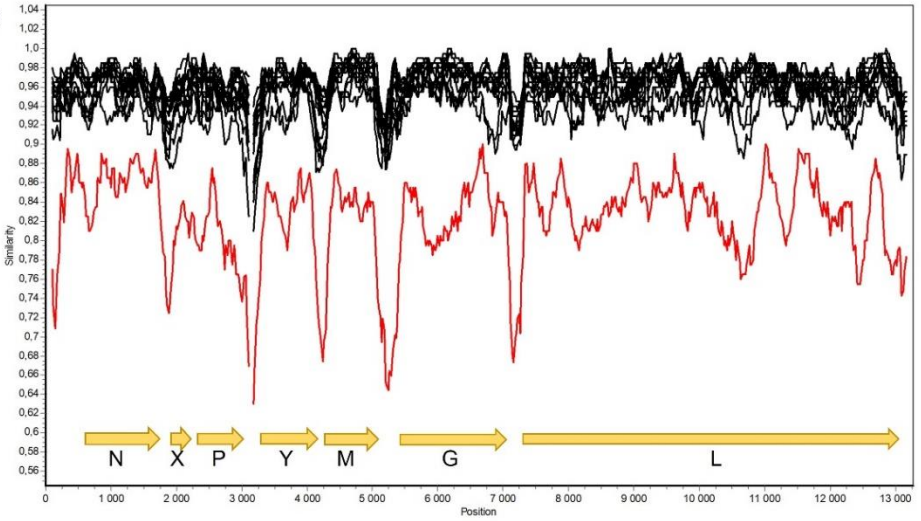


Fig. 3-2. Phylogenetic tree inferring relationships of 29 Physostegia chlorotic mottle virus (PhCMoV) isolates (among which were 21 new genomes published in this study) based on nucleotide alignment of near-complete genomic sequences. The phylogenetic tree was inferred using the maximum likelihood method (GTR + G + I model) based on the full genome sequence MUSCLE alignment (nucleotides) of all the PhCMoV isolates known at this date. Each isolate is labeled with its name and the information of the collection: country (flag), host, and year. Orange squares and letters highlight identical collection sites (farm). The values on the branches show the percentage of support out of 1,000 bootstrap replications, and the scale bar indicates the number of nucleotide substitutions per site.

a



b

		Full genome	N	X	P	Y	M	G	L
PhCMoV isolates genome and ORF size range	nts	13320	1269-1482	306-366	885	864-1104	756-798	1857-1935	5841-5874
	aa	na	422-494	101-121	294-295	287-368	251-265	618-656	1938-1958
Range of sequence identity of Nd_SL1 to all known PhCMoV	nts ID (%)	82	85-87	76-81	79-81	83-85	84-85	82	83-84
	aa ID (%)	na	96-100	73-76	83-87	92-99	93-97	91-94	95-96
Range of sequence identities amongst all known PhCMoV isolates excluding Nd_SL1	nts ID (%)	>93	>94	>89	>93	>93	>95	>94	>94
	aa ID (%)	na	>95	>88	>95	>95	>96	>97	>98

Fig. 3-3. Differences and similarities between selected Physostegia chlorotic mottle virus (PhCMoV) isolates in different open reading frames (ORFs). A, graphic representation of nucleotide identities (%) using SIMPLOT of 16 full genome sequences of PhCMoV (ref query = KX636164; Window: 200 bp, Step: 20 bp; Gapstrim: On; Hamming across the complete genome sequence and its genome organization). In red is the representation of the most divergent isolate Nd_SL1. B, nucleotide and amino acid sequence identities calculated for N, X, P, Y, M, G, and L ORFs for all isolates studied. The identities (%) were calculated based on MUSCLE alignment (Muscle 3.8.425 by Robert C. Edgar). The number of base pairs for the full genome sequence is indicated for KX636164.

Discussion

By collaborating and sharing data before submitting the results for publication, eight European research groups investigated Physostegia chlorotic mottle virus in detail and characterized its genome and biology.

This study demonstrates the ability of PhCMoV to naturally infect seven host plants (annual and perennial ones) in addition to the two previously known hosts across seven families including, economically important crops (S. lycopersicum, S. melonga, C. sativus), newly introduced crops in Europe (I. batatas, S. affinis), wild plants (G. paviflora, P. maculosa) and ornamentals (Helleborus sp). Similar observations have

been made for other alphanucleorhabdoviruses, e.g., EMDV with more than 25 hosts recorded on CABI (2021) (https://www.cabi.org/), including crops and perennial plants such as Hibiscus sp., Hydrangea macrophylla, Agapanthus or Pittosporum sp. (CABI, 2021). This suggests that the host range of PhCMoV is likely to be wider than described here, and additional perennial hosts might help the virus overwinter.

Our results outline PhCMoV symptomatology on a large range of plants collected in fields, gardens, and greenhouses. Overall, the presence of the virus was associated with virus-like symptoms on leaves (vein clearing, chlorosis, mottling...) and severe symptoms on fruit (deformation, marbling, uneven ripening). Only two samples (S. affinis and P. maculosa) did not exhibit any symptom, suggesting that asymptomatic plants might host the virus. We did not describe the symptomalogy of PhCMoV on sweet potato because of co-infection. Considering only the samples single infected with PhCMoV, the symptoms were often variable across plants from the same species. These variations may be due to several biases. First, they could be due to human perception since different people recorded the symptoms. Secondly, the plants corresponded to different cultivars and were grown under heterogeneous conditions. In addition, symptom expression may be different depending on the plant growth stage at the time of infection. Nevertheless, the presence of the virus was always associated with obvious vein clearing on the leaves of G. paviflora, eggplant and Helleborus. This symptom was also described for EMDV on honeysuckle and eggplant (Martelli et al., 1987).

The severe symptoms observed on tomato fruit (marbling, mottling, uneven ripening) confirmed previous reports (Table 3-1, Gaafar et al., 2018, Vučurović et al., 2021). Even though remarkable, these symptoms were not specific to PhCMoV: similar observations were made in the case of other viral infections (EMDV, (Blancard, 2009) pepino mosaic virus (Hanssen et al., 2009), tomato brown rugose fruit virus (EPPO Bulletin, 2020)) and in the case of nutrient disorder mostly referred as “blotchy ripening” (Adams et al., 1995). The symptoms observed on tomato leaves were highly variable (mottling and vein clearing) and sometimes absent. Therefore, tomato leaves do not represent a good indicator of PhCMoV presence.

Vein clearing was observed on the leaves of four out of five eggplant samples. Vein clearing is not specific for PhCMoV as it is also representative of the presence of EMDV and alfalfa mosaic virus (Martelli et al., 1986; Sofy et al., 2021) but it is generally associated with viral presence on eggplant and can differentiate viral presence from that of other pathogens, abiotic stress, or nutritional disorders. Interestingly, this symptom can be used to monitor the spread of the virus in a parcel infected by PhCMoV. Finally, the number of samples per species sampled on the other host plants was too low to be associated with a specific symptom.

To confirm the presence of PhCMoV and to study its mechanical transmission, infected leaves collected in various sites were mechanically inoculated on different

indicator hosts. In total, four out of eighteen indicator plant species were successfully inoculated and showed systemic symptoms (Table 3-2; D. metel, D. stramonium, N. benthamiana, N. occidentalis P1). In the previous studies, C. quinoa, N. occidentalis '37B', N. clevelandii, N. tabacum 'WB', Physalis floridana were also mechanically inoculated (Table 3-2; Menzel et al., 2018; Gaafar et al., 2018). This host range is similar to the one of EMDV which includes: N. clevandii, N. glutinosa, N. rustica, N. tabacum, P. hybrida, and P. floridana (Mavrič et al., 2006; Katis et al., 2011). No systemic symptom of EMDV infection has ever been reported on C. quinoa and D. stramonium. Despite the overall high sequence identity of the PhCMoV isolates analysed in this study, the results were variable across laboratories. Some plants were successfully inoculated in some laboratories but not in others (for example: N. occidentalis P1, D. stramonium) and the range of observed symptoms on a same host plant species was variable. Inoculation success and symptom expression depend on environmental conditions (Hull, 2014) and inoculum sources. In addition, at NPPO-NL, some symptoms were recorded four to seven weeks post-inoculation on N. occidentalis P1 and N. benthamiana which is longer than the recommended period of three weeks (Roenhorst et al., 2013). Indexing is very important to maintain and study viruses in controlled conditions, to separate them in case of multiple infection and to find the best host for virus purification. It would also be interesting to inoculate several plant species in the same experimental conditions to compare the impact of divergent isolates on symptom expression. Overall, all the studies converged toward N. benthamiana being the best experimental PhCMoV host. Our study also showed that inoculated plants suspected to host PhCMoV should be kept in a greenhouse for symptom observations for at least seven weeks.

With the generation of 29 sequences of near-complete genome, PhCMoV is now the plant rhabdovirus with the highest number of near-complete genomes available. These genomes provided data for studying the virus genetics in relation to host range, geographical location, and time. Despite genetic variability ranging between 82% and 100% of nt sequence identity (for the near-complete genome), the 29 samples did not cluster according to country or host plant.

In addition, there was 100% identity between isolate KY706238 collected on tomato in 2003 and isolate Ge_CS1 collected on cucumber from the same site in 2020. This genome conservation over time was observed in four distinct sites across Europe (yellow boxes in Fig. 3-2). It suggests that the genome of PhCMoV does not evolve rapidly once established in a suitable ecosystem. This highlights the impact of the geographical dimension on the genetic evolution of PhCMoV and is in line with observations on other plant rhabdoviruses (EMDV, RSMV) whose phylogenetic clusters correlate with geographical localization, but not necessarily with the host plant or the sampling date (Tang et al., 2014; Yang et al., 2018; Pappi et al., 2015). Since plant rhabdoviruses are transmitted from plant to plant by insects in a persistent and propagative manner and no other way of natural transmission is known, insect

vectors are likely to be the cause of the strong selective pressure on the genetic diversity of plant rhabdoviruses (Power, 2000).

For the 29 isolates analysed in this study and collected from eight countries and eight host plant species, the genetic diversity was very low (less than 3% at the nt level for the near-complete genome). This low genetic diversity has been observed in other plant rhabdoviruses. For example, Yang et al., (2018) showed that the genome of 13 isolates of rice stripe mosaic virus (RSMV) collected in various geographical regions in China showed 99.4% of nucleotide sequence identity. In another study, Samarfard et al., (2018) showed a 99% aa sequence identity of protein N across 13 alfalfa dwarf virus (ADV) isolates from different regions in Argentina. In our study, between 92 and 99 % of nt sequence identity was observed among the 29 available PhCMoV genomes with only one outlier, Nd_SL1, with 81-82% of nt sequence identity with the other 28 isolates (Fig. 3-3). However, this isolate was not an outlier at the protein level; for instance, it had more than 96% aa identity with all the PhCMoV isolates for protein N, while the nt sequence identity ranged between 85 and 87% for the corresponding gene. Similar observations have been reported for the cytorhabdovirus lettuce necrotic yellows virus (LNYV): the ORF encoding protein N of two subgroups were approximatively 80% identical at the nt level and 96% identical at the aa level (Higgins et al., 2016).

Overall, this study brings together some key elements on the genetic diversity of PhCMoV and its potential drivers. It shows the importance of accumulating genomic sequences from diverse isolates to draw robust conclusions. Viral genomes from samples of different origins (new location, new host, or different collection date) support a better understanding of the genetic diversity and evolution of this virus, but the presence of an exception (i.e. isolate Nd_SL1) suggests that the genetic diversity of PhCMoV remains partly uncovered and that the results need to be interpreted carefully. Considering the severity of the symptoms observed on economically important crops, it is unclear why the virus remained unnoticed for at least the past two decades. The lack of appropriate diagnostic tests might be one of the reasons for this delay, since cross-reactions occurred with one of the EMDV antibodies in 2008. This suggests that additional infections may have been misdiagnosed. In addition, samples collected in 2002 (Fr_SM1), 2008, 2011 (Fr_SL1), 2013 (Fr_SM2/3) and 2014 (Fr_SL2) were set aside for identification because the PCR products showed 78% nt identity with EMDV and the PhCMoV sequence was not available at the time. Our research highlights the strength of HTS in plant virus detection, and the wider application of these technologies for virus detection might explain the sudden simultaneous identifications throughout Europe. Another complementary hypothesis of the recent detections might be that the virus was present in the environment, but went unnoticed because it did not cause a problem (low incidence), and a recent change in the environment led to its emergence. Whether the virus is more prevalent nowadays or whether it was overlooked in the past remains unknown. However, the current situation requires rapid characterization and a common response from

European countries because simultaneous PhCMoV detections in several European countries over a wide host range including economically important foodstuffs suggests that the virus could be an emerging pathogen. In that context, pre-publication data sharing, and collaboration have been valuable to improve knowledge about this virus and would be beneficial in the future to efficiently evaluate the risk associated with any emerging disease and implement management strategies.

One of the next priorities will be to identify the insect vector and its life cycle. EMDV, PYDV and CYDV are the closest relatives of PhCMoV with a known vector, and those vectors all belong to Cicadellidae, which makes leafhoppers prime candidates for transmitting PhCMoV (Dietzgen et al., 2021). Furthermore, according to the transmission tests carried out by Babaie et al., (2003) EMDV was transmitted by one specific leafhopper (Agallia vorobjevi) and not by the other 13 leafhopper species present in and around EMDV-infected fields. This suggests specific virus-insect transmission. A second priority line of research will be to determine in which hosts the virus is present in winter. This ability of plant rhabdoviruseses to infect different host plants across families is an important factor to be considered for controlling the disease because a large diversity of plants can serve as a reservoir during the no-crop season. A third axis will be to assess the impact of the virus in terms of yield and economical loss on different cultivars and when the plants are inoculated at different developmental stages.

Finally, understanding the epidemiology of the virus and the reasons for its multiple recent detections in Europe are key elements to be investigated in order to evaluate if it can present a threat for vegetable production and how to prevent potential outbreaks.

Acknowledgments

The authors would like to thank Pier de Koning (NPPO) for his help to analyse the data and Katarina Bačnik and Olivera Maksimović Carvalho Ferreira (NIB) for assisting in sample collection. We also acknowledge Laurent Minet (Hortiforum asbl / Centre Technique Horticole de Gembloux) for spotting the first symptoms of the virus in Belgium, monitoring the site thoroughly and providing expert advice and Frederic Dresen (ULIEGE) for his strong technical support, advices and insightful guidance throughout the study. We are grateful for the support of the growers and farmers who allowed us to access their properties and collect samples over multiple years.

Funding

European Union´s Horizon 2020 Research and Innovation program under the Marie Sklodowska-Curie, Grant Agreement no. 813542. Federal public service, public health, Belgium, Grant Agreement no. RT 18/3 SEVIPLANT. Euphresco project

'Phytosanitary risks of newly introduced crops (PRONC), Grant Agreement no. 2018-A-293. European Union's Horizon 2020 research and innovation programme, Grant agreement No. 871029

Supplementary materials

Access to the supplementary materials:
https://apsjournals.apsnet.org/doi/suppl/10.1094/PDIS-12-21-2800-RE

Literature cited

Adams, P. and Ho, L.C. 1995. Uptake and distribution of nutrients in relation to tomato fruit quality. Acta Hortic. 412, 374-387

Adams, I. P., Fox, A., Boonham, N., Massart, S., and De Jonghe., K. 2018. The impact of high throughput sequencing on plant health diagnostics. Eur J Plant Pathol. 152:909–919.

Alfaro-Fernández, A., Córdoba-Sellés, C., Tornos, T., Cebrián, M. C., and Font, M. I. 2011. First Report of Eggplant mottled dwarf virus in Pittosporum tobira in Spain. Plant Disease. 95:75–75.

Babaie, G., and Izadpanah, K. 2003. Vector Transmission of Eggplant Mottled Dwarf Virus in Iran. Journal of Phytopathology. 151:679–682.

Bejerman, N., Dietzgen, R. G., and Debat, H. 2021. Illuminating the Plant Rhabdovirus Landscape through Metatranscriptomics Data. Viruses. 13:1304.

Blancard. 2009. Les maladies de la tomate. Quae, pages 346-366.

Dietzgen, R. G., Bejerman, N. E., Goodin, M. M., Higgins, C. M., Huot, O. B., Kondo, H., et al. 2020. Diversity and epidemiology of plant rhabdoviruses. Virus Research. :197942.

Dietzgen, R. G., Bejerman, N. E., Mei, Y., Jee, C. L. J., Chabi-Jesus, C., Freitas-Astúa, J., et al. 2021. Joá yellow blotch-associated virus, a new alphanucleorhabdovirus from a wild solanaceous plant in Brazil. Arch Virol. 166: 1615–1622.

Dolja, V. V., Krupovic, M., and Koonin, E. V. 2020. Deep Roots and Splendid Boughs of the Global Plant Virome. Annual Review of Phytopathology. 58:23–53.

Fox, A. 2020. Reconsidering causal association in plant virology. Plant Pathology. 69:956–961.

Fraile, A., and García-Arenal, F. 2016. Environment and evolution modulate plant virus pathogenesis. Curr Opin Virol. 17:50–56.

Gaafar, Y., Richert-Pöggeler, K., Maaß, C., Vetten, H.-J., and Ziebell, H. 2019. Characterisation of a novel nucleorhabdovirus infecting alfalfa (Medicago sativa). Virology Journal. 16.

Gaafar, Y. Z. A., Abdelgalil, M. a. M., Knierim, D., Richert-Pöggeler, K. R., Menzel, W., Winter, S., et al. 2018. First Report of physostegia chlorotic mottle virus on Tomato (Solanum lycopersicum) in Germany. Plant Disease. 102:255–255.

Gaafar, Y. Z. A., Westenberg, M., Botermans, M., László, K., De Jonghe, K., Foucart, Y., et al. 2021. Interlaboratory Comparison Study on Ribodepleted Total RNA High-Throughput Sequencing for Plant Virus Diagnostics and Bioinformatic Competence. Pathogens. 10:1174.

Hammond, J., Adams, I. P., Fowkes, A. R., McGreig, S., Botermans, M., Oorspronk, J. J. A. van, et al. Sequence analysis of 43-year old samples of Plantago lanceolata show that Plantain virus X is synonymous with Actinidia virus X and is widely distributed. Plant Pathology. 70: 249– 258.

Hanssen, I. M., Paeleman, A., Vandewoestijne, E., Bergen, L. V., Bragard, C., Lievens, B., et al. 2009. Pepino mosaic virus isolates and differential symptomatology in tomato. Plant Pathology. 58:450–460.

Higgins, C. M., Chang, W.-L., Khan, S., Tang, J., Elliott, C., and Dietzgen, R. G. 2016. Diversity and evolutionary history of lettuce necrotic yellows virus in Australia and New Zealand. Arch Virol. 161:269–277.

Hou, W., Li, S., and Massart, S. 2020. Is There a "Biological Desert" With the Discovery of New Plant Viruses? A Retrospective Analysis for New Fruit Tree Viruses. Front. Microbiol. 11: 2953

Hull, 2014, Plant virology, Elsevier, Fith edition, Chapt, Page 171

Jackson, A. O., Dietzgen R. G., Goodin M. M., Bragg J.N., Min Deng, M. 2005. Biology of Plant Rhabdoviruses. Annual Review of Phytopathology. 43:1, 623-660 Pathology 152 (4): 909-19.

Jones, R. A. C., Boonham, N., Adams, I. P., and Fox, A. 2021. Historical virus isolate collections: An invaluable resource connecting plant virology's pre-sequencing and post-sequencing eras. Plant Pathology. 70:235–248.

Katis, N., Chatzivassiliou, E., Clay, C. M., Maliogka, V., Pappi, P., Efthimiou, K., et al. 2011. Development of an IC-RT-PCR assay for the detection of Eggplant mottled dwarf virus and partial characterization of isolates from various hosts in Greece. Journal of plant pathology. 93:253–362.

Kuhn, J. H., Adkins, S., Alioto, D., Alkhovsky, S. V., Amarasinghe, G. K., Anthony, S. J., et al. 2020. 2020 taxonomic update for phylum Negarnaviricota (Riboviria: Orthornavirae), including the large orders Bunyavirales and Mononegavirales. Arch Virol. 165:3023–3072.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 35:1547–1549.

Martelli, G. P., and Cherif, C. 1987. Eggplant Mottled Dwarf Virus Associated with Vein Yellowing of Honeysuckle. Journal of Phytopathology. 119:32–41.

Martelli, G. P., and Hamadi, A. 1986. Occurrence of eggplant mottled dwarf virus in Algeria. Plant Pathology. 35:595–597.

Massart, S., Candresse, T., Gil, J., Lacomme, C., Predajna, L., Ravnikar, M., et al. 2017. A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies. Front. Microbiol. 8: 45.

Mavrič, I., Tušek Žnidarič, M., Viršček Marn, M., Dolničar, P., Mehle, N., Lesemann, D.-E., et al. 2006. First report of Eggplant mottled dwarf virus in potato and tomato in Slovenia. Plant Pathology. 55:566–566.

Menzel, W., Richert-Pöggeler, K., Winter, S., and Knierim, D. 2018. Characterization of a nucleorhabdovirus from Physostegia. Acta Horticulturae. 1193:29–38.

Oñate-Sánchez, L., and Vicente-Carbajosa, J. 2008. DNA-free RNA isolation protocols for Arabidopsis thaliana, including seeds and siliques. BMC Research Notes. 1:93.

Pappi, P. G., Dovas, C. I., Efthimiou, K. E., Maliogka, V. I., and Katis, N. I. 2013. A novel strategy for the determination of a rhabdovirus genome and its application to sequencing of Eggplant mottled dwarf virus. Virus Genes. 47:105–113.

Power, A. G. 2000. Insect transmission of plant viruses: a constraint on virus variability. Current Opinion in Plant Biology. 3:336–340.

Roenhorst, J. W., Botermans, M., and Verhoeven, J. T. J. 2013. Quality control in bioassays used in screening for plant viruses. EPPO Bulletin. 43:244–249.

Samarfard, S., Bejerman, N. E., and Dietzgen, R. G. 2018. Distribution and genetic variability of alfalfa dwarf virus, a cytorhabdovirus associated with alfalfa dwarf disease in Argentina. Virus Genes. 54:612–615.

Sofy, A. R., Sofy, M. R., Hmed, A. A., Dawoud, R. A., Refaey, E. E., Mohamed, H. I., et al. 2021. Molecular Characterization of the Alfalfa mosaic virus Infecting Solanum melongena in Egypt and the Control of Its Deleterious Effects with Melatonin and Salicylic Acid. Plants. 10:459.

Tang, J., Elliott, C., Ward, L. I., and Iqram, A. 2014. Identification of Eggplant mottled dwarf virus in PEQ Hibiscus syriacus plants imported from Australia. Australasian Plant Dis. Notes. 10:6.

Tomato brown rugose fruit virus. 2020. EPPO Bulletin. 50:529–534.

Verhoeven, J. T. J., and Roenhorst, J. W. 2000. Herbaceous test plants for the detection of quarantine viruses of potato*. EPPO Bulletin. 30:463–467.

Vučurović, A., Kutnjak, D., Mehle, N., Stanković, I., Pecman, A., Bulajić, A., et al. 2021. Detection of Four New Tomato Viruses in Serbia using Post-Hoc High-Throughput Sequencing Analysis of Samples from a Large-Scale Field Survey. Plant Disease. 105:9, 2325-2332.

Whitfield, A. E., Huot, O. B., Martin, K. M., Kondo, H., and Dietzgen, R. G. 2018. Plant rhabdoviruses—their origins and vector interactions. Current Opinion in Virology. 33:198–207.

Yang, X., Chen, B., Zhang, T., Li, Z., Xu, C., and Zhou, G. 2018. Geographic Distribution and Genetic Diversity of Rice Stripe Mosaic Virus in Southern China. Front. Microbiol. 9: 3068.

Chapter 4

Biological characterization of an emergent virus infecting vegetables in diversified production systems: physostegia chlorotic mottle virus

Temple, C., Blouin, A.G., Boezen, D., Botermans, M., Durant, L., et al., Biological characterization of an emergent virus infecting vegetables in diversified production systems: physostegia chlorotic mottle virus. Submitted to Phytopathology in 2023

Abstract

In 2018, Physostegia chlorotic mottle virus (PhCMoV) was discovered in Austria in a *Physostegia* plant. Subsequent collaborative efforts established a link between the presence of PhCMoV and severe fruit symptoms on important crops like tomato, eggplant, and cucumber across nine European countries. Nevertheless, specific knowledge gaps, which are crucial to assess the potential risks the virus can pose for the production and how to manage it, remain to be adressed. In this study, the transmission mode, prevalence, and disease severity of PhCMoV were investigated. The investigation mapped out the historical and geographic footprint of the virus, spanning back 30 years and including a new country, Switzerland. Bioassays in greenhouse demonstrated PhCMoV can result in up to 100% tomato yield losses depending on the phenological stage of the plant at the time of infection. PhCMoV was found to naturally infect 11 new host plant species across seven families, extending the host range of PhCMoV to 20 plant species across 14 plant families. The study also identified a polyphagous leafhopper species (*Anaceratagallia*) as a natural vector of PhCMoV. Overall, PhCMoV was widespread in small-scale diversified vegetable farms in Belgium where tomato is grown in soil, occurring in approximately one-third of such farms. However, outbreaks were sporadic and it can be suggested that they were associated with the cultivation of perennial plants in tomato tunnels that can serve as a reservoir host for the virus and its vector. To further explore this phenomenon and manage the virus, studying the ecology of the vector would be beneficial.

Keywords: Physostegia chlorotic mottle virus, host range, symptoms, field experiment, greenhouse assay, yield loss, prevalence, transmission, leafhoppers

Introduction

Application of high throughput sequencing (HTS) technologies enabled the first identification of Physostegia chlorotic mottle alphanucleorhabdovirus (PhCMoV) from *Physostegia virginiae* (*Lamiaceae*) in 2018 (Menzel et al., 2018). PhCMoV is a rhabdovirus which belongs to the *Alphanucleorhabdovirus* genus, and more precisely, to a cluster that includes eggplant mottle dwarf virus (EMDV), potato yellow dwarf virus (PYDV), constricta yellow dwarf virus (CYDV) and joá yellow blotch-associated virus (JYBaV) (Dietzgen et al., 2021). PhCMoV is most closely related to EMDV.

After re-analyzing historical samples, the presence of PhCMoV was confirmed from samples collected in 2002 (Temple et al., 2021). With 29 isolates sequenced, PhCMoV is the plant rhabdovirus with the most near-complete genomes available to date (Temple et al., 2021). Furthermore, genomic studies showed that although genetic variability ranged between 82 and 100% of nucleotide sequence identity (for the near-complete genome), PhCMoV showed a very low genomic variation in the same environment for a long period (17 years) on different annual host plants (Temple et al., 2021).

HTS has significantly improved knowledge of plant viral diversity, and the evolution of known viruses, as well as enabling the discovery of new plant viral species (Bejerman et al., 2020, Bejerman et al., 2021, Adams et al., 2018, Lefeuvre et al., 2019). However, genomic information alone does not provide enough indications to assess the phytosanitary risks associated with novel plant viruses and to develop appropriate management strategies to control epidemics (Massart et al., 2017). Therefore, it is necessary to study the biology and epidemiology of a new virus to understand its potential risk for crops and wild plants. In 2017, a framework was published to help with the evaluation of biosecurity, commercial, regulatory, and scientific impacts of new viruses that need to be characterized for an efficient risk assessment (Massart et al., 2017). This framework is currently under revision to focus the research on the association between the presence of the virus earlier (Fontdevila et al., 2023). The revised framework will follow the suggestions put forward by Fox (2020) : to optimize the study of symptomology caused by plant viruses while still being reliable by combining experimental data with epidemiological observations, statistical analysis, and testing of asymptomatic and symptomatic plants in the field. Afterwards, if the novel virus is still considered a threat to crop production, it is recommended to continue the virus characterization by filling the remaining knowledge gaps related to its genetic diversity, geographic distribution, prevalence, severity, host range, symptom causality and transmission mode.

Studying the transmission mode of a new virus and its vectors is one of the most important tasks to understand how to limit the spread of a virus. Yet, it is one of the least-studied criteria, as shown for tomato and fruit tree viruses (Hou et al., 2021, Rivarez et al., 2021). Furthermore, research on the transmission mode for new viral species is laborious and require a lot of time and resources. For example, transmission tests require to start and maintain colonies of potential insect vector candidates in

appropriate control conditions. In that context, reviewing close virus relative vectors can greatly narrow the range of insect to test. Looking for the presence of insects in infected areas or being attentive to the distribution of the virus in the field is important to identify the mode of transmission. In Dietzgen et al., (2015), phylogenetic studies based on the protein L homology of various plant rhabdoviruses showed that these viruses clustered according to their insect vector type. PhCMoV cluster with EMDV, PYDV and CYDV, which are transmitted by leafhoppers while other plant rhabdovirus can also be transmitted by planthoppers, aphids, mites and whitefly (Dietzgen et al., 2020). A large study on the vector of EMDV in Iran revealed its transmission by the leafhopper *Agallia vorobjevi* (Dlab.) after testing different arthropods species, including two mites, one psyllid, one thrips, five aphids, four planthoppers and 14 leafhoppers species found on EMDV infected sites. The transmission of a "cucumber isolate of EMDV" by leafhopper *(Anaceratagallia laevis* (Ribaut) and *Anaceratagallia ribauti* (Ossiannilsson)) was also demonstrated in France with better efficiency for *A. laevis* (Della Giustina et al., 2000). Two strains of PYDV were described based on their differential transmission by the leafhopper vector *Anaceratagallia sanguinolenta* (PYDV) and *Agallia constricta* (CYDV). These results suggest that the vector of PhCMoV is likely to be a specific specie of leafhopper close to the *Anaceratagallia* or *Agallia* genus.

In 2021, pre-publication data sharing between scientists resulted in an international collaboration and the first evaluation of the risk associated with PhCMoV. This evaluation, combined with previous reports, highlighted the importance of PhCMoV, because its sudden detection in multiple European countries was shown to be associated with severe symptoms on economically important crops such as tomato, eggplant and cucumber (Gaafar et al., 2018; Vučurović et al., 2021, Temple et al., 2021). The study extended the known natural host range of PhCMoV to nine different plant species (seven families) across nine European countries. PhCMoV was associated with severe symptoms on the fruits and with vein clearing on the leaves. Subsequently, in Belgium, where multiple occurrences of the virus was recorded, 2,100 asymptomatic tomato plants were screened from 21 vegetable farms with soil-grown tomatoes on for the presence of viruses. No detection of PhCMoV was recorded, while the virus was detected in six of the sites on symptomatic plants, reinforcing the exisiting association between virus presence and symptom development on field (Temple et al., submitted).

The aim of this publication is to better study the biology of PhCMoV in order to refine the analysis of the phytosanitary risks it poses and to propose management measure to limit its spread. The biological characterization focuses on filling knowledge gaps related to prevalence and epidemiology, disease severity, transmission modes, host range and symptomology as suggested in a recent optimized scientific and regulatory framework for their characterization and risk analysis (Fontdevila et al., 2023).

Material and methods

1.1. Sampling and laboratory tests

Selection of the best sampling tissue for tomato

For three different tomato cultivars ('Black cherry', 'St Jean d'Angely' and 'Trixi') from site A (Supplementary table 1), a specific sampling on seven different tissues per plant was carried out. At the lower part of the plant, (1) an old leaf (6^{th} from the bottom), (2) the first re-growth, (3) a mature fruit and (4) a re-growth at middle height was sampled. Then, (5) the apex, (6) the uppermost fruit (not mature) and the (7) uppermost mature fruit was sampled as well (Fig. 4-1). Finally, for the cultivar 'St Jean d'Angely' and 'Trixi', (8) a leaf from average age, taken from the middle height of the plant was also collected. Symptoms on each of the samples were recorded.

For the cultivar 'Black cherry', five asymptomatic plants (AS), ten plants that only showed symptoms at the bottom of the plant (S) and ten plants that showed systemic symptoms (S++) were selected. The seven different samples were collected on each plant as described in Fig. 4-1.

Two asymptomatic plants were selected for the two other cultivars ('St Jean d'Angely' and 'Trixi'), while six and seven symptomatic plants were selected for the cultivar 'St Jean' and 'Trixi', respectively. The samples were tested by ELISA to evaluate the best tissue to sample for detecting the virus.

Plants and insects sampling

Testing the presence of PhCMoV in symptomatic plants

During summer, tomato and eggplant crops were visually inspected for the presence of PhCMoV suspicious symptoms (tomato unven ripened and deformed fruits and eggplants with vein clearing on new leaves). All the symptomatic plants were counted, collected and frozen at -20°C. If a PhCMoV-suspicious symptomatic tomato or eggplant was spotted in a site, particular attention was given to the presence of viral-like symptoms (vein clearing, mosaic, deformation, dwarfing) on the other plants species present on the site. The suspected virus-infected plants were pictured, sampled and tested by RT-PCR. The samples were collected as part of a survey on tomatoes grown on soil dedicated to the fresh market in the Walloon Region of Belgium in 2020, 2021 and 2022. In total, 27 farms were surveyed with five of them visited over two consecutive years. The number of plants per species, year and site is indicated in Supplementary table 1.

Testing the presence of PhCMoV on new host plants

Two distinct ecological large-scale plant virome surveys in the Netherlands, collected wild plant species, including *Anthriscus sylvestris*, *Solanum nigrum*, *Viola arvensis*, *Geranium molle* and *Hypericum perforatum*. Specimens were sampled, irrespectively of symptoms in 2020 and 2021. Between 3 and 20 plants per species

were collected and pooled before virus detection was performed using HTS of total RNA.

Detection of PhCMoV in historical samples

Five samples of tomato and one sample of cucumber kept in an historical collection of plant samples stored frozen (-20°C) and labeled as "rhabdovirus" were reexaminated. The samples were collected in Switzerland (Tessin, Zurich and Valais) between 1993 and 2006. They were tested for the presence of PhCMoV by RT-PCR and the oldest tomato sample (collected in 1993, accession 3216 at Agroscope, Nyon, Switzerland) was sequenced by HTS of total RNA.

Insects trapping

In the site A, leafhoppers belonging to the *Anaceratagalliae*, *Eupteryx*, and *Euscelidius* genera were observed in October 2021 around symptomatic sorrel (*Rumex acetosa)* plants. The specimens were collected from these plants, and from the walls of the plastic greenhouse with an insect-aspirator.

Laboratory testing

RNA extraction from plants

The protocol used for RNA extraction of historical samples was described in Reynard et al., 2022. For the Belgian samples (survey and transmission experiments), RNA extraction was carried out following the protocol described Onate-Sanchez and Vicente-Carbajosa (2008). For samples of *A. sylvestris* and *S. nigrum* RNA was extracted from about 1 g frozen leaf tissue, according to Botermans et al., (2013). For *V. arvensis*, *G. molle* and *H. perforatum*, RNA was extracted using the Maxwell RSC Plant RNA Kit (Promega).

DNA and RNA extraction from insect

The entire insect body was ground using a micro-pestle in 1.5 mL Eppendorf tubes filled with 0.5 ml TRIzol™ (Invitrogen®). Half a ml of TRIzol™ was then added to the samples. After overnight incubation at room temperature, 200 µl of chloroform was added. Each tube was then vortexed for 15 seconds, incubated at room temperature for 3 minutes and centrifuged for 15 minutes at 12.000 *g* and 4 °C. RNA present in the aqueous phase (supernatant) was precipitated in 500 µl of isopropanol before 10 minutes of incubation at 4°C and centrifugation at 12,000 g and 4°C. Next, the supernatant was removed, and pellets were washed twice in 1 ml of fresh 75% ethanol. At each wash, tubes were spun for 5 minutes at 7,500 *g* and 4°C. After the last wash, the remaining ethanol was removed by pipetting and air drying. RNA was resuspended in 30 µl of sterile water. DNA present in the inferior phase was precipitated in 300 µl of 100% ethanol. Tubes were mixed by inversions and incubated for 3 minutes at room temperature before centrifugation for 5 minutes at 2,000 *g* and 4°C. The supernatant was removed, and pellets were washed twice in 1 ml of 0.1M sodium citrate in 10% ethanol for 30 minutes. At each wash, tubes were centrifuged for 5 minutes at 2,000 *g*, and 4°C and the supernatant was discarded. After pipetting away any residual drops, DNA was resuspended in 30 µl of sterile water.

Detection of PhCMoV by HTS

Extracted RNA of the historical accession 3216 and a plant used for mechanical inoculation in control conditions (named "GH24") was processed using the protocol described for Be_GP1 in Temple et al., 2021 prior to Illumina sequencing (total RNA and ribodepletion). RNA of *Anthriscus sylvestris* and *Solanum nigrum* were also analyzed using a protocol based on total RNA and ribodepletion prior to Illumina sequencing, as described for Nd_SL1 in Temple et al., 2021. Finally, for *Viola arvensis*, *Geranium molle* and *Hypericum perforatum*, RNA extracts were subjected to ribodepletion and cDNA synthesis as described in Liefting et al. (2021). The cDNA was sequenced using the Illumina NovaSeq platform. Reads were trimmed using fastp (default settings) (Chen et al. 2018) and assembled using rnaviralspades (default settings) (Meleshko et al., 2021). PhCMoV genomes were detected using blastn with using the nt reference database (Altschul, 1990).

Detection of PhCMoV by RT-PCR and ELISA

RNA extracts were reverse transcribed in cDNA prior to PCR using the primers and PCR conditions according to Gafaar et al., 2018.

ELISA tests were performed using PhCMoV antibodies JKI-2051 (kindly provided by Heiko Ziebell, JKI), at a dilution of 1:2000 (v/v). The protocol of Clark et Adams (1977) was followed.

DNA barcoding for insect identification

The subsequent amplification step of the PCR was performed using MangoTaq™ DNA Polymerase (Bioline, Belgium) and the primers LCO1490 and HCO2198 designed by Folmer et al., (1994) and the following cycling conditions: 94°C for 1 min, 35 cycles of 94°C for 15 sec, 48°C for 20 sec, 72°C for 20 sec and a final extension step of 3 min at 72°C. The amplified products were purified with the QIAquick PCR purification kit (QIAGEN), and amplicons were sent to Macrogen Europe lab (Amsterdam) for Sanger sequencing. Finally, sequences obtained with forwards and reverse primers were two by two de novo assembled on Geneious Prime® 2020.0.5 software for each sample. Primer sequences were removed and resulting consensus sequences were analyzed using BLASTn and default settings. The identification of the insect was validated when the percentage of identity was higher than 95% with a given reference sequence.

1.2. Prevalence and symptom association studies on farm

Prevalence of PhCMoV in tomato in Wallonia

The prevalence of plants with PhCMoV-like symptoms was estimated by visual inspection for each site, by dividing the number of tomato plants showing PhCMoV symptoms by the total number of tomato plants. We used the prevalence of symptoms as a proxy for virus prevalence.

Association between PhCMoV presence and symptoms on eggplants

To understand better the correlation between the PhCMoV-like symptoms (vein clearing and deformations on new leaves) and the presence of the virus in eggplant, 13 symptomatic plants from the cultivar 'Shakira' (Supplementary Fig. 1) and 109 asymptomatic eggplants surrounding the symptomatic plants were sampled. This collection was conducted on the site C (Supplementary table 1) at the end of August 2020 where the presence of the virus was confirmed the previous year (Temple et al., 2021). The distribution of the symptomatic plants was mapped in the greenhouse (Supplementary Fig. 1). In the greenhouse, 440 eggplants were grown, and most symptomatic plants (11/13) were located near the entrance with only two additional eggplants showing symptoms on the first row, near an opening in the middle of the tunnel (Supplementary Fig. 1). The samples were analyzed by ELISA. The 13 symptomatic and the 48 asymptomatic plants immediately surrounding the symptomatic ones, were tested individually, whereas the 61 asymptomatic plants situated away from the symptomatic plants were tested in pools of two to ten plants.

Association between PhCMoV presence and symptoms on several tomato cultivars

In site A (Supplementary table 1), tomato plants showing symptoms on fruits (deformations, uneven ripening) and leaves (vein clearing on re-growth) were observed in October 2020. In the greenhouse, 14 different tomato cultivars were grown, with approximately 120 plants per cultivar. Half of the plants were planted in April, and the other half in June. In total, 116 symptomatic tomato plants were mapped (Supplementary Fig. 2). Whenever possible, at least three symptomatic plants per cultivar were collected and tested by ELISA for the presence of PhCMoV. In total, 61 plants showing symptoms were tested. Ten asymptomatic plants per cultivar were collected and pooled by five to test by ELISA. The 55 other plants showing the same symptoms were considered positive to calculate the virus prevalence for each cultivar (Supplementary table 2).

1.3. Greenhouse inoculations

The PhCMoV isolate GH24 from tomato was reactivated on *N.benthamiana* before being used for inoculation. The studied plants were mechanically inoculated in greenhouse by gently rubbing the leaves with 0.02M potassium phosphate buffer (pH 7,4) with 0.2% sodium diethyldithiocarbamate or 2% of polyvinylpyrrolidone freshly added for the evaluation of the impact on yield and carborundum. After 5 minutes, the leaves were rinsed under tap water.

Expanding knowledge on PhCMoV host range and symptomology

To confirm the PhCMoV host range and to evaluate the associated symptoms, 12 different plants species (*Capsicum annum, Tropaleum majus, Lavatere trimestris, Stachys affinis, Galinsoga pavirflora, Cucumis sativus, Ipomea purpurea, Nicotiana*

glutinosa, Nicotiana benthamiana, Petunia x hybrida, S. melongena, S. lycopersicum) including two different cultivars of tomatoes ('Suzy' and 'Black cherry') were mechanically inoculated. The number of inoculated plants per species/cultivars varied between 5 and 20 and is indicated in Table 4-1. Symptoms were monitored seven to ten weeks post-inoculation and the samples were tested by ELISA for the presence of PhCMoV.

Evaluation of the impact of PhCMoV on the yield and quality of tomatoes

To study the impact of PhCMoV on yield and quality, two cultivars of tomato ('Black cherry' (BC) and 'Cupidissimo F1' (CU) were mechanically inoculated with PhCMoV (GH24) at three different developmental stages: 4 weeks after sowing (BC-1 and CU -1), 8 weeks after sowing (BC-2 and CU -2), and 14 weeks after sowing (BC-3 and CU -3). These different time points were chosen because 1) the first one (4 weeks after sowing) corresponded to the control laboratory conditions and the stage when tomato plants are usually inoculated for indexing, 2) Eight weeks after sowing corresponds approximatively to the tomato developmental stage at which growers plant the seedlings in the greenhouse (the moment they can potentially get infected), 3) 14 weeks after sowing correspond to the flowering stage. The cultivar 'black cherry' was chosen because it seemed highly sensitive to the virus in the field. The cultivar 'Cupidissimo F1' was chosen because it seemed less sensitive and belonged to another type of tomato ('Coeur de boeuf'). Two dwarf tomato cultivars ('Tom Thumb' and 'Micro Tom') were also inoculated at one time point (3,5 weeks after sowing).

For the inoculation at the ~4-weeks stage, only one leaf per plant was inoculated with 1mL of inoculum solution. For the inoculation at the 8-weeks and 14-weeks stages, three newly formed leaves per plant were inoculated with 1mL of the inoculum solution per leave. At the different time points, between 2 and 5 plants were "inoculated" only with the buffer solution as a negative control. The number of plants inoculated with PhCMoV at the different time points was 20, 18 and 16 for 'Black cherry', 15, 19 and 9 for the cultivar 'Cupidissimo' and 14 for the two dwarf cultivars (Supplementary table 3).

The plants were randomly distributed in a greenhouse, and after the first inoculation, they were visually inspected for symptoms each week. When the fruits reached maturity, they were harvested, weighed and classified as suitable for the market (asymptomatic) or not (symptomatic, showing deformations, marbelling or anomalies of coloration, Fig. 4-2). At the end of the experiment (when most of the plants were starting to die), re-growth or symptomatic tissues (fruit or leaves) were sampled and tested by ELISA to confirm the presence of PhCMoV. If a negative result was given on an asymptomatic plant inoculated, another organ (bottom fruit) was tested to confirm the absence of the virus. Only ELISA positive plants were considered for statistical analyses.

The total weight of marketable and non-marketable fruit was calculated for each plant. Then, the total marketable weight of the plants inoculated at the different time points was compared to the mock-inoculated condition using the Wilcoxon test on R

Studio software. A significance threshold of 0.05 was used when testing for differences between control and inoculated plants at each time point.

Vector investigation

Transmission assays

Since *Anaceratagallia* sp. represented the best candidate for the transmission of PhCMoV, two transmission assays were designed with the collected specimens. For the first assay, 10 *Anaceratagallia* leafhoppers captured as described before in site A (2.2.4) were fed on various host plants infected with PhCMoV (eggplant, *Galinsoga* sp, tomato, sorrel) for 20 days in an insect-proof cage (Temperature: 21°C, Humidity: 50%, Day:night: 16:8). After that, one specimen (LF43-3) was transferred to a healthy eggplant seedling (TR47). Another one (LF43-4) was transferred to a healthy tomato seedling (TR52). After four days, the leafhopper on TR47 died and was stored at -20°C. After 13 days, LF43-4 was transferred to another healthy tomato seedling (TR62) for 24h before being stored at -20°C. The plants were grown in insect-proof empty cages and tested by RT-PCR for the presence of PhCMoV seven weeks after the first contact with the leafhopper. DNA and RNA of the two insects was extracted for species identification by DNA barcoding and PhCMoV testing.

For the second assay, six *Anaceratagallia* leafhoppers were collected on the same site (A) near infected plants and directly transferred on three healthy tomatoes and three healthy eggplant seedlings for the second assay. All the plants were tested for the presence of PhCMoV by RT-PCR. Dead insects were collected and stored at -20°C before DNA/RNA extraction and DNA barcoding/PhCMoV testing. One insect was lost during the process.

Morphological identification

In summer 2022, one *Anaceratagallia* male specimen was caught in site A using the process as in 2021. First, its genital parts were dissected and pictured to morphologically identify the specimens (Supplementary Fig. 3). For this purpose, the classification Key of Tishechkin et al., 2020 was used. Then, DNA was extracted as described above for COI barcoding identification.

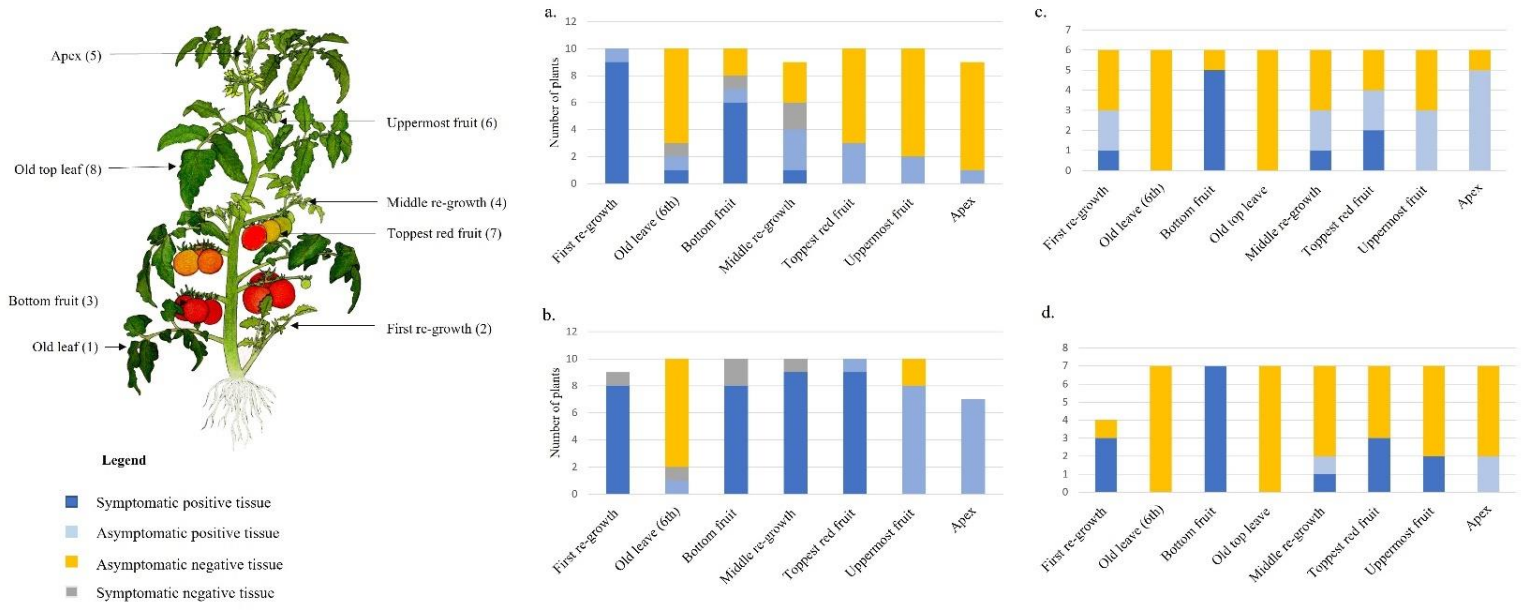


Figure 4-1. Detectability of PhCMoV in different tissues by ELISA a) Cultivar 'black cherry' with mild symptoms, b) Cultivar 'black cherry' with severe symptoms, c) Cultivar 'St Jean d'Angely', with medium symptoms d) Cultivar 'Trixi', with medium symptoms. The status of the plant (positive or negative) was assessed by ELISA

Figure 4-2. Mean of total yield (green + red color), marketable yield (green color) and unmarketable yield (red color) per tomato plant of the 'Black cherry' cultivar (a) and 'Cupidissimo F1' cultivar (b) when the plants were infected at three time points. Infected-1: 4 weeks after sowing, infected-2: 8 weeks after sowing, infected-3: 14 weeks after sowing, mock: control plants inoculated with the buffer only, c) Represent pictures of tomato considered as « marketable » (asymptomatic) which corresponds to the green color, d) Represent pictures of tomato considered as « unmarketable » (symptomatic), which corresponds to the red color, n= number of plants per conditions, Asterisks indicate statistically significant differences of sealable fruits compared with the mock-treated plants (**: p-value <0.01, *** p-value <0.001)

Results

1.4. Selection of the most appropriate tissue for PhCMoV detection

In site A, special attention was given to 'Black cherry', 'St Jean d'Angely' and 'Trixi' to assess the distribution of the virus in tomato plant and the best tissues to sample to detect the virus. The seven plant samples of the nine asymptomatic tested plants were tested negative by ELISA for PhCMoV. At least one of the seven sample tested per plant classified as "symptomatic" was positive. For the plants 'Black cherry' that showed mild symptoms, PhCMoV was best detected in symptomatic lower re-growth and symptomatic lower fruits (Fig. 4-1). When plants showed severe symptoms, the virus was detected in the upper parts, whether they were symptomatic (bottom fruit, middle re-growth, topped mature fruit) or not (uppermost fruit, apex). The symptomatic bottom fruit (4) was the most reliable sample in the positive plants of 'St Jean' and 'Trixy' (Fig. 4-1). Overall, most positive tissues exhibited symptoms, but some detections were also made on asymptomatic tissues, mainly situated at the top of the plant, especially for the cultivar St Jean d'Angely (Fig. 4-1). All the positive plants' oldest tissues (6th old leave, old middle leave) were asymptomatic and

negative. Overall, symptomatic fruits or re-growth at the bottom of the plants seemed to be the best tissues to observe PhCMoV symptoms in various tomato cultivars and to detect the virus.

1.5. PhCMoV was already present in Europe in 1992

Six symptomatic historical samples from Switzerland, dating back to 1992 were tested positive for PhCMoV. The confirmation of the presence of PhCMoV in Europe is therefore set back by more than a decade and in a new country. The genome of the sequenced sample was deposited on Genbank (accession OQ689795).

1.6. Identification of new host plants and symptomatology

During the field survey, eleven new plant species were identified as natural host for PhCMoV, extending the number of PhCMoV known host plant species from nine to twenty. It includes *A. sylvestris, Chenopodium album, Capscium annuum, G. molle, H. perforatum, Malva sylvestris, Physalis peruviana, Rumex acetosa, S. nigrum, Tropaeolum majus,* and *V. arvensis*. Four of them belong to two plant families already known to host PhCMoV (*Polygonaceae* and *Solanaceae*) and seven other plant species belong to new families: *Amaranthaceae, Apiaceae, Geraniaceae, Hypericaceae, Malvaceae, Tropaeolaceae,* and *Violacea.* When PhCMoV was detected through HTS, the sequences were deposited in Genbank (accession number: OQ716531, OQ716532, OQ716533, OQ318170, OQ318171).

Vein clearing and deformations were observed on leaves of some of the host plants identified in Belgium (*C. album, C. annuum, M. sylvetris, P. peruviana, R. acetosa, T. majus*, Supplementary Fig. 4). However, it is impossible to assess whether the symptoms were caused by PhCMoV, other viruses or abiotic stress since the presence of other viruses in mixed infection cannot be excluded and no information was collected for putative abiotic stresses for these plants.

1.7. Symptoms causality of PhCMoV on its hosts

To study the association between the presence of PhCMoV and symptoms on different host plants, *C. annum, T. majus, L. trimestris, S. affinis, G. parviflora, C. sativus, I. purpurea,* and *S. melogena* were mechanically inoculated with GH24 (accession OQ689794) under greenhouse conditions. Four additional species were used as positive control (*N .glutinosa, N. benthamiana, Petunia x hybrida, S. lycopersicum*). HTS and bioinformatic analyses confirmed that the original plant used for inoculation was only infected by PhCMoV (isolate GH24). Almost all the control plants (62/68) were successfully inoculated and showed symptoms of vein clearing, deformation and yellowing (Table 4-1, Fig. 4-3). For *T. majus* and *L. trimestris*, two plants out of 15 were successfully inoculated by PhCMoV (Table 4-1). Infected *L. trimestris* plants showed weak vein clearing on some of the leaves, while the symptoms on *T. majus* were more visible (vein clearing, leaf deformation) and resemble the one observed on the field (Fig. 4-3). Three out of five plants of *S. affinis* were successfully inoculated, and the plants showed vein clearing and discolouration (Fig. 4-3), in contrast with the symptomless *S. affinis* collected in the field and sequenced previously (accession MZ322957, Temple et al., 2021).

Figure 4-3. Symptoms of PhCMoV on leaves of different plant species mechanically inoculated by GH24. a. *Tropaleum majus*, b. *Stachys affinis,* c. *Nicotiana glutinosa,* d. *Nicotiana benthamiana,* e. *Petunia x hybrida,* f. *Lavatere trimestris,* g. *Solanum melongena*

	GH24	
Inoculated test plant	**Symptoms**	**ELISA/ RT-PCR**
N. glutinosa	vc, d	4/10
N. benthamiana	vc, d, y	9/9
Petunia hybrida	vc, d	9/9
C. sativus 'Belt alpha'	-	0/10
C. annuum 'Yolo wonder'	-	0/10
S. lycopersicum 'Suzy'	vc, d	20/20
S. lycopersicum 'Black Cherry'	vc, d	20/20
Stachis affinis	vc, m, y	3/5
Tropaeolum majus 'Girerd'	vc, d	2/15
Lavatere trimestris	y, vc, lln	2/15
Galinsonga pavirflora	-	0/15
Ipomea purpurea 'Grandpa Ott'	-	0/15
Solanum melongena 'tsakoniki'	vc, d	3/4

Table 4-1. Mechanically inoculated plant species with PhCMoV (isolate GH24), symptoms observed and RT-PCR results. Legend: m = mottle, vc = vein clearing, d= deformation, y= yellowing, lln = lesions locales nécrotic, - = asymptomatic

1.8. Association of PhCMoV with symptomatic eggplants

In site C, 13 symptomatic plants showing vein clearing and deformations on the new leaves or all the leaves and 109 asymptomatic eggplants were collected in a tunnel and tested for PhCMoV (Supplementary Fig. 1). The ELISA results indicated that the 13 symptomatic samples were positive, and in 108 asymptomatic plants surrounding the symptomatic ones, the virus was not detected. Only one asymptomatic plant situated next to a symptomatic plant was positive and showed symptoms on the next visit.

1.9. PhCMoV detection on different tomato cultivars

In site A, 118 tomato plants belonging to 12 different cultivars showed symptoms of PhCMoV. These plants were distributed on both sides of the greenhouse independently of the plantation date. Still, although the same cultivars were planted on both sides, the number of symptomatic plants was much when planted in April (75/900) than in June (24/900) Supplementary Fig. 2.

All the 61 symptomatic plants tested by ELISA were positive for the virus while the 140 asymptomatic plant pools were negative for all the 14 cultivars. These results suggested that the virus presence is well associated with the presence of similar symptoms on various cultivars. In the greenhouse, the presence of symptomatic and positive plants of *R. acetosa* was also mapped (Supplementary Fig. 2).

The most impacted cultivar was 'Black cherry' as 48% of the plants showed symptoms, followed by the cultivar 'Gipsy noir', 'Gustafano F1', 'St Jean d'Angely' and 'Trixi', where between 5 and 10% of the plants were symptomatic. On the other hand, no detection of the virus and no symptomatic plants were recorded for the cultivar 'Charlie's green' and 'Suzy'. Finally, the prevalence of symptomatic plants was below 4% of total plants for the other seven cultivars (Supplementary table 2).

1.10. Prevalence of PhCMoV in Belgian farms

During field surveys conducted in two Belgian provinces on vegetable farms dedicated to local-market, the presence of PhCMoV was confirmed by RT-PCR on all symptomatic host plant tested (*S. lycopersicum, S. melongena, G. parviflora, C. sativus, S. affinis, C. album, C. annuum, M. sylvetris, P. peruviana, R. acetosa, T. majus*) when observed in nine out of 27 farms (33%) (Fig. 4-4, Supplementary table 1).

Five farms where PhCMoV was detected were visited the following years and the presence of the virus was confirmed each time (Supplementary table 1). In site A and C, the virus was detected on symptomatic plants during three consecutive years.

1.11. Prevalence within the farms based on tomato symptoms observations

In the nine farms infected by PhCMoV, the prevalence of tomato with PhCMoV-like symptoms was used as a proxy for evaluating the virus prevalence. It was demonstrated through field and greenhouse assays that the association between the presence of PhCMoV and symptoms on tomato fruits (deformations, uneven ripening) was strong, suggesting that disease symptoms are a good proxy for virus infection.

In most farms (7/9), less than 1.5% of the tomato plants were infected at the collection date (Fig. 4-4). The symptomatic plants were mainly distributed at the tunnels' entrances or near openings. In two sites (A and P), the prevalence of the virus in tomato reached 7% and 13%, respectively (Fig. 4-4). While weeds and other annual plants than tomato were commonly present in most of the visited greenhouse, the culture of perennial plants (sorrel, strawberry, aromatics…) was noticed inside tomato tunnels only in site A and P (Supplementary table 1).

In site P, 85 and 200 tomato plants (belonging to 20 cultivars) were grown into two side-by-side small tunnels (4x30m) and the symptomatic plants were mainly observed in one of the two tunnels (38/85 tomato plants exhibited PhCMoV symptoms). In the other tunnel, only 2/200 plants were symptomatic.

After 2021, the producers of site P removed all the perennial plants and weeds that were present in the highly infected tunnel. The following year (2022), the presence PhCMoV in the tunnel was only sporadic (only 2-3 tomato plants were showing the

symptoms) while the same annual crops were cultivated (tomato, capsicum and cucumber). A similarly low number of PhCMoV infected eggplants was observed outdoors in the same two seasons (2021 and 2022).

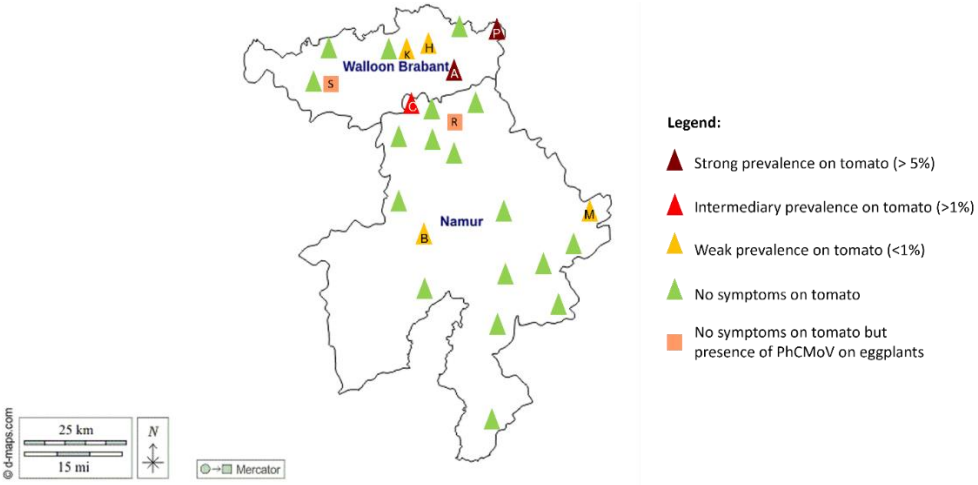


Figure 4-4. Distribution and « prevalence » of PhCMoV based on symptoms observations in tomato and eggplant (R, S) in the province of Walloon Brabant and Namur (Belgium). The « prevalence » was calculated based on the number of PhCMoV-symptomatic tomato plants divided by the total number of tomato grown in a site (Supplementary table 6)

1.12. Yield assay

To study the impact of the virus on yield, tomato plants ('Black cherry' (BC), n=54 and 'Cupidissimo F1' (CU), n=43) were inoculated at three different developmental stages. Overall, the global inoculation success rate one was higher for BC than CU (87% vs 63%), but infection was always above 50% for each time point and each cultivars (Supplementary table 3). This rate did not decrease with the plant age for the two cultivars (Supplementary table 3).

For BC, the first symptoms following the first inoculation time point was spotted on leaves approximatively 8 weeks post inoculation (wpi) (Supplementary table 3). They were mostly found on fruits for the second and third inoculation time points (Supplementary table 3) approximatively eight and 15 wpi respectively.

For CU, the first symptoms following the first inoculation were spotted on leaves and fruit at the same time, approx. 9.5 wpi. After the second inoculation, symptoms were observed more often on fruit than on leaves at approx. 14 wpi, and those of the third inoculation were all spotted first on fruit approx. 10 wpi (Supplementary table 3).

It is important to note that for both cultivars, the number of weeks before the appearance of the first symptoms was very variable from one plant to another in a

same time point (e.g. symptoms can be observed 4 wpi or 22 wpi for the second time point in CU) and the indicated number is the median (supplementary table 3).

For both cultivars, total asymptomatic fruit weight was significantly reduced when plants were innoculated at four weeks after sowing and eight weeks after sowing compared to the control (Fig. 4-2, Supplementary table 4). However, the difference was no longer significant when comparing plants that were infected 14 weeks after sowing. The average yield from asymptomatic fruits (marketable fruits) per plant decreased by 99% and 65% for the BC infected at the first and second inoculation time point (Fig. 4-2). This drop was mainly due to a reduction in the number of fruits per plant for the first time point, which reached 0 for some of the plants and due to the presence of symptoms on the remaining fruits (Supplementary table 4). For the second time point, the number of asymptomatic fruits was higher than for the first time point (close to 50%) (Fig. 4-2).

The same phenomenon was observed for cultivar CU although yield reduction at the first and second time point compare to the control was less drastic than for BC (Fig. 4-2).

1.13. Insect identification and PhCMoV transmission

Leafhoppers belonging to *Anaceratagallia* genus and present on one of the two most affected sites (A) were collected and used in transmission tests to test if they could transmit PhCMoV.

In the first experiment, the two *Anaceratagallia* leafhoppers (LF43-3 and LF43-4) that fed on infected PhCMoV tomato and eggplant in cages successfully transmitted the virus to two healthy seedlings (TR47 and TR62). The plants were tested positive for PhCMoV by RT-PCR seven weeks after their contact with the viruliferous insects. PhCMoV was also detected in the insect body of the two insect specimens, despite the fact that one had been feeding on a healthy plant for the last 14 days before its death. Only the infected status of one plant (TR52), which was also in contact with the infected *Anaceratagallia* leafhopper (LF43-4), was inconclusive, as the plant was nearly dead before the RNA extraction process.

Comparison of the COI sequence of the two leafhoppers which have transmitted PhCMoV (LF43-3 and LF43-4) with the NCBI database matched with the accession OK275083 "*Anaceratagallia* sp.", which has not be identified at the species level with 95% identity (id) (Supplementary table 5).

In the second trial, six additional *Anaceratagallia* leafhoppers were directly put from the field onto six healthy seedlings in a cage (three eggplants and three tomatoes). After four weeks, two eggplants were showing vein clearing on new leaves. The symptoms appeared on the third eggplant after two more weeks and on two tomato plants eight weeks after the first contact with the leafhoppers. These five symptomatic plants (out of six) were tested positive for PhCMoV. Dead leafhoppers were collected 10 and 23 days after being in contact with the plants and one of them (LF42b) was tested positive for PhCMoV. COI barcoding and sequence homology with the NCBI database was also performed to identify the five remaining insect

species. Two specimens (LF42-a and LF42-e) matched to accession OK205264 (98% id) and MZ631325 (100%id) respectively, namely "*Anaceratagallia lithuanica",* and one specimen (LF42-b) matched the unnamed specimen of *Anaceratagallia* (OK275083, Supplementary table 5). The results remained inconclusive for two other specimens.

Finally one year after the transmission test, a new *Anacertagallia* specimen was collected for morphological identification. According to the classification key of Tchechekin, 2020, the specimen was *A. fragariae* (Supplementary Fig. 3). However, the COI sequence matched with the accession OK205264 (98% id) which was labeled as *A. lithuanica*. The COI sequence was deposited on GenBank (accession: OQ469522).

Discussion

With this study, PhCMoV is now known to be present in ten European countries. Since its first detection in 2018, the virus was detected in diseased plants from economically important crops such as tomato, eggplant and cucumber, highlighting the importance of better understanding its biology (Temple et al., 2022). The framework for the evaluation of biosecurity, commercial, regulatory, and scientific impacts of new viruses revised by Fontdevila et al., (2023) was followed to fill the knowledge gaps required to understand the phytosanitary risks associated with PhCMoV.

By investigating symptomatic historical samples, PhCMoV was detected in 30-year-old samples from Switzerland, where it had not previously been identified. In parall, eleven new species have been added to the virus' natural host range, bringing to 20 the number of plant species susceptible to PhCMoV from 14 plant families. These findings suggest that the actual natural host range is probably much wider, given the diversity of the host range identified in four years. This biological aspect is coherent with EMDV, the closest virus to PhCMoV, which includes more than 25 hosts recorded on CABI (2021) (https://www.cabi.org/). Perennial and biennial hosts such as *A. sylvestris*, *R. acetosa* or *S. affinis* could allow PhCMoV to overwinter.

In order to study symptoms causality, bioassays were performed in controlled conditions for some selected host plants. All the successfully infected plants showed symptoms (72 plants from 12 different plant species). The association of PhCMoV with symptoms on *T. majus* and *L. trimestris* which belong to two families not previously known to host PhCMoV (*Tropaeolaceae and Malvaceae*) was assessed, and deformation and vein clearing symptoms were observed. Mechanical inoculations of PhCMoV induced discolouration and yellowing on the leaves of *S. affinis*, in contradiction to our initial field observation (Temple et al., 2022). Environmental conditions and host genotype may explain this difference, given that inoculation is carried out under conditions that are optimal for the development of virus symptoms in the greenhouse (Hull, 2014).

In contrast, symptoms observed in tomato and eggplant in controlled conditions were identical to those observed in the field (uneven-ripened and deformed fruits, vein clearing and deformed leaves, dwarfing and shortened nods for the most impacted

plants). For these two host plants, all four criteria to assess symptoms causality described by Fox (2020) were fulfilled: 1-experiment : the symptoms observed in control conditions after mechanical inoculation were similar to the ones observed in the field; 2-Strength: numerous symptomatic host plants showing the same symptoms as in the experiments and asymptomatic plants were tested in a virus-infected plot and demonstrated the association of symptoms with the presence of PhCMoV; 3-consistency: symptoms observation caused by PhCMoV were consistent on several occasions, in different geographical regions and on successive years; improving the 4-coherence and plausibility. In this study, the results suggest that a tomato plant must exhibit symptoms on at least one tissue to be tested positive. Additionally, there is a higher probability of observing symptoms on the lower organs (such as lower fruits or re-growth) compared to the upper organs for some tomato cultivars.

Although the association between PhCMoV and the presence of symptoms is strong on eggplant and tomato, symptoms can be mistaken with other plant viruses such as alfalfa mosaic virus for eggplant and with tomato brown rugose fruit virus (ToBRFV), pepino mosaic virus (PepMV) or tomato fruit blotch virus for tomato, although, none of these viruses were confused with PhCMoV as part of our work (Ciuffo et al., 2020; Temple et al., 2022). ToBRFV and PepMV have very different biological properties compared to PhCMoV. These viruses are highly transmissible through contact and by seeds, can remain stable in the environment and represent therefore a major threat for tomato production (Oladokun et al., 2019, Hanssen et al., 2010). ToBRFV is considered a quarantine pest in Europe (A2 list, EPPO) and requires strict sanitation measures and obligatory notification in case of detection. Therefore, making a correct diagnosis through laboratory testing in case of PhCMoV-like symptoms in tomato remains crucial.

The symptoms caused by PhCMoV can also be confused with those of EMDV in eggplant, tomato, cucumber and capsicum, but these two viruses have the same mode of transmission and the same management strategy should therefore be applied (El Maataoui et al., 1985, Roggero et al., 1995). However, with the exception of the South of France, these two viruses are present in disctinct area, EMDV is endemic in the Mediterranean basin, where it is widespread (CABI), while PhCMoV is so far mostly detected in temperate European countries.

Assessment of the severity of PhCMoV on tomatoes showed that the time of inoculation is strongly influencing its impact on plants. In our experiments, plants infected before the planting date (eight weeks after sowing) showed a total loss of marketable fruit yield for one of the two cultivars tested (‘Black cherry’) and a ~75% drop for the second (‘Cupidissimo F1’). Yield loss was mainly caused by a degradation of the fruit appearance, a reduction in the number of fruits per plant, and a decrease in average fruit weight. A preliminary study on short-lived tomato cultivars ('Tom Thumb' and 'Micro-Tom') showed a similar trend in yield loss (Durant 2021). In the present study, the impact on yield was, however, reduced when ‘Black cherry’ and ‘Cupidissimo F1’ were inoculated at a later developmental stage. Similar observation were reported with turnip mosaic virus on cabbage, where early inoculation significantly reduced the number and quality of marketable harvested

plants compared to later inoculation (Spence et al., 2007). This was also observed in tomato infected by tomato yellow leaf curl virus (TYLCV) as plant age at inoculation had a significant reduced effect on yield loss (Levy et Lapidot, 2007). Conversely, in chard (*Beta vulgaris* subsp. *vulgaris*) infected with beet mosaic virus, or tomato infected with PepMV, late infection had the most pronounced effects on non-marketability (Spence et al., 2006, Spence et al., 2007). In addition, we did not measure an increased resistance of mature plants to infection through mechanical inoculation, and the decrease in yield measured was likely due to the long latent phase. Indeed, for the plants infected at the latest time point (14 weeks), symptoms appeared after the harvest peak (median of 15 and 10 weeks post inoculation). These results underline the importance of safeguarding plants from PhCMoV infection during the early developmental stages.

Overall, PhCMoV was detected in one-third of the visited diversified farms where vegetables are grown in soil in Belgium. In addition, once the virus was detected in a farm, it was systematically detected the following year (for the five sites that were re-visited), suggesting the persistence of the virus in the environment. However, the prevalence of the virus in the field was very limited (<1%) in all but two sites, where the virus was problematic (prevalence >7%). The presence of perennials in direct vicinity of tomatoes in tunnels was noted in the two most affected sites (A and P) and could account for the high virus pressure. This was confirmed by the drastic reduction in the incidence of PhCMoV observed in site P between 2021 and 2022after the elimination of all perennials and weeds in a tomato tunnel.

The spread of a viral disease is mainly driven by the ability of the vector (if any) to transmit the virus between plants (Whitfield et al., 2018). Two distinct species of the *Anaceratagalliae* genus were isolated from cultivated sorrel (*R. acetosa*) in site A: *A. fragariae* identified morphologically and an unidentified *Anaceratagallia* sp (only sequenced and not enough specimens to perform a full morphological identification). Based on their COI sequences, these two species were previously described at a same site on a wild strawberry plant (*Fragaria vesca*) in the Czech Republic, suggesting they co-habits (Fránová et al, 2021). Tishechkin 2020 has reviewed the taxonomy of the *Anaceratagalliae* genus based on the shape of male genitalia and revealed that multiple synonyms has been erroneously described for this genus. He suggested that the species of *A. lithuanica* was synonymous to *A. ribauti* and very similar in morphological traits and in ecological preferences to *A. fragariae*. Specimens morphologically identified in this study as *A. fragariae* had a COI sequenced almost identical to an accession recorded as *A. lithuanica* suggesting that *A. lithuanica* was misidentified with *A. fragariae* and incorrectly named in the GenBank database.

The transmission of PhCMoV was only demonstrated for the unidentified species of the *Anaceratagalliae* genus. Nevertheless, it is not excluded that *A. fragariae* can also transmit PhCMoV. In a transmission assay, Giustina et al., (2000) demonstrated the transmission of "an EMDV strain" by two different *Anaceratagallia* species, with a better efficiency for *A. laevis* than *A. ribauti*. However, since the diagnosis for the

virus was only based on symptoms observation and this experiment was contradicted by Babaie et Izadpanah (2003) who showed that *A. laevis* does not transmit EMDV, this is questioning whether Giustina et al., 2000 could have investigated PhCMoV instead of EMDV, since we now know that the two viruses coexist in this region and their symptoms are very similar. If confirmed, the unidentified main vector of PhCMoV would be *A. laevis*.

Overall, it is crucial to identify the vector of PhCMoV at species level and to investigate if multiple *Anaceratagallia* species can transmit the virus. Many aspects of the ecology and behavior of *Anaceratagallia* are lacking, and the epidemiology of plant rhabdoviruses is strongly influenced by their specific insect vectors in which they also replicate (Hogenhout et al., 2003, Whitfield et al., 2018). Thereafter, studying the ecology and behaviour of PhCMoV vectors will provide a better understanding of the emergence of the disease and could account for the sudden multiple detections of PhCMoV after decades of unnoticed presence. This work will also make it possible to develop more appropriate management strategies specifically targeting plants that are suitable for the reproduction of the vector or serve as winter habitats. The ability to rear these leafhoppers will also greatly accelerate the research as, it is impossible to morphologically differentiate species among living individus and females. This would also permit to test the transovarial vertical transmission of PhCMoV, as this was demonstrated with wheat yellow striate virus, another alphanucleorhabdovirus (Du et al., 2020). In this study, we observed that *A. fragariae* can mate, reproduce and complete a full lifecycle on *R. acetosa* in the laboratory as shown by Tishechkin, 2020. In addition, one adult specimen was observed crawling on a cultivated sorrel in the middle of winter (January 2022) in one of the greenhouse of site A, suggesting the potential role of this plant in the overwintering of the leafhoppers, and the possibility of the adult to survive the winter as previously described for multiple *Anaceratagallia* species (Nickel and Remane, 2002). Regarding their behaviour, our observations also revealed that *Anaceratagallia* leafhoppers were not very mobile. This was also supported by the distribution of the virus in farms, generally in patch, often close to the entrance of the tunnels. The proximity of plants in which they can mate and complete a full life cycle near by annual plants may contribute to the development of the disease.

Overall, one of the explanation of the sudden detection of PhCMoV in European temperate area where EMDV has never been reported can rely on agricultural practices. There has been an increase in the number of producers in Belgium who are cultivating a wide range of plant species (20-45) over a limited area (< 2.5 ha) (Dumont et Baret, 2017). The virus was mainly detected in this type of structure where producers often promote sustainable farming, diversity, natural regulation of pests and contact with their consumers, such as Community Supported Agriculture (Temple et al., 2023, Dumont et Baret, 2017, Boeraeve et al., 2020, Tamburini et al., 2020). Exchanges between natural ecosystems and cultivated plants or between different cultivated plant species are more common than in close and highly controlled greenhouses and might favour the presence of plant viruses in cultivated plants and

pathogen spillovers, which is considered the first step of virus emergence (Elena et al., 2014).

Notably, extensive monitoring of tomato viruses in the Netherlands' industrial production systems (which utilize insect-proof glasshouses) did not identify the presence of PhCMoV in 125 production sites (data not shown). This suggests that the virus could have, to date, no impact on commercial-scale industrial tomato production.

To conclude, this work makes PhCMoV one of the best characterized new tomato viruses after ToBRFV. All the steps of the optimized scientific and regulatory framework for the characterization and risk analysis of a new virus (Fontdevila Pareta et al., 2023) were compiled and almost all the characterization criteria proposed by Rivarez and colleagues (2021) are now met. Overall, this plant rhabdovirus can pose a threat to tomato and other vegetable crops in small, diversified farms. However, with a better understanding of its biology and agricultural practices, management measures can be proposed to mitigate an epidemic. The benefits of this work result in an efficient initial management solution to answer growers problems with PhCMoV on tomato grown under tunnel as shown in site P. Further knowledge on the vector of PhCMoV will help predict potential epidemics and develop improved management strategies.

Acknowledgments:

The authors would like to warmly thank Frederic Dresen from the Phytopathology laboratory of Gembloux-Agro-Biotech (University of Liege) for providing invaluable technical assistance during the experimentation process, including greenhouse assays and transmission tests. Additionally, the authors acknowledge his valuable guidance in designing the experiments, improving mechanical inoculation techniques, and sharing his expertise on plant viruses, characterization of new viral species, and rearing and capturing leafhoppers.

Catherine Wipf-Scheibel (INRAE), Nathalie Dubuis (Agroscope) and Elisabeth Demonty (CRA-W) are also thanks for their important support in the laboratory analyzes.

We thank Prof. Frederic Francis from the Entomology department of Gembloux Agro-Biotech (University of Liege) for making space available in the insect rearing chambers of his laboratory and for letting CT managing independently leafhoppers' rearing.

The authors express their gratitude to Nuria Fontdevila, Johan Rollin and Julien Ponchard, for assisting in sample collection and weighing the fruits.

We would also like to thank Heiko Ziebell from Julius Kuhn Institute (JKI) for kindly providing the PhCMoV antibodies. Finally, we are very grateful for the support of the growers who allowed us to access their properties and collect samples over multiple years, and multiple times, especially grower A, to whom we went so many times to catch leafhoppers and to sample plants.

Data, scripts, code, and supplementary information availability

All the sequences were deposited on GenBank (accessions: OQ689794, OQ689795, OQ716531-OQ716533, OQ318170 and OQ318171).

Supplementary data are available in open access: https://zenodo.org/record/7849861#.ZEe0y2hMTIU

Funding

European Union´s Horizon 2020 Research and Innovation program under the Marie Sklodowska-Curie, Grant Agreement no. 813542.

Federal public service, public health, Belgium, Grant Agreement no. RT 18/3 SEVIPLANT 55.

References

Adams, I. P., Fox, A., Boonham, N., Massart, S., and De Jonghe, K. 2018. The impact of high throughput sequencing on plant health diagnostics. Eur J Plant Pathol. 152:909–919.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J Mol Biol. 215:403–410.

Babaie, G., and Izadpanah, K. 2003. Vector Transmission of Eggplant Mottled Dwarf Virus in Iran. Journal of Phytopathology. 151:679–682.

Bejerman, N., Debat, H., and Dietzgen, R. G. 2020. The Plant Negative-Sense RNA Virosphere: Virus Discovery Through New Eyes. Front Microbiol. 11.

Bejerman, N., Dietzgen, R. G., and Debat, H. 2021. Illuminating the Plant Rhabdovirus Landscape through Metatranscriptomics Data. Viruses. 13:1304.

Blancard, D. 2009. Les maladies de la tomate - Identifier, connaître, maîtriser. Librairie Quae : des livres au coeur des sciences.

Boeraeve, F., Dendoncker, N., Cornélis, J.-T., Degrune, F., and Dufrêne, M. 2020. Contribution of agroecological farming systems to the delivery of ecosystem services. Journal of Environmental Management. 260:109576.

Botermans, M., van de Vossenberg, B. T. L. H., Verhoeven, J. Th. J., Roenhorst, J. W., Hooftman, M., Dekter, R., et al. 2013. Development and validation of a real-time RT-PCR assay for generic detection of pospiviroids. Journal of Virological Methods. 187:43–50.

Chen, S., Zhou, Y., Chen, Y., and Gu, J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 34:i884–i890.

Ciuffo, M., Kinoti, W. M., Tiberini, A., Forgia, M., Tomassoli, L., Constable, F. E., et al. 2020. A new blunervirus infects tomato crops in Italy and Australia. Arch Virol. 165:2379–2384.

Clark, M. F., and Adams, A. N. Y. 1977. Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses. Journal of General Virology. 34:475–483.

Dietzgen, R. G., Bejerman, N. E., Goodin, M. M., Higgins, C. M., Huot, O. B., Kondo, H., et al. 2020. Diversity and epidemiology of plant rhabdoviruses. Virus Research. :197942.

Dietzgen, R. G., Bejerman, N. E., Mei, Y., Jee, C. L. J., Chabi-Jesus, C., Freitas-Astúa, J., et al. 2021. Joá yellow blotch-associated virus, a new alphanucleorhabdovirus from a wild solanaceous plant in Brazil. Arch Virol. 166:1615–1622.

Du, Z., Fu, Y., Liu, Y., and Wang, X. 2019. Transmission Characteristics of Wheat Yellow Striate Virus by its Leafhopper Vector Psammotettix alienus. Plant Disease. 104:222–226.

Dumont, A. M., and Baret, P. 2017. Classifications et cartographies des systèmes de production et commercialisation des légumes frais en Région wallonne (Belgique).7

Durant., Master thesis, 2021, University of Liège, Gembloux Agro-biotech, http://hdl.handle.net/2268.2/13255

El Maataoui, M., Lockhart, B. E. L., and Lesemann, D. E. 1985. Biological, serological, and cytopathological properties of tomato vein-yellowing virus, a rhadbovirus occurring in tomato in Morocco. Phytopathology (USA).

Elena, S. F., Fraile, A., and García-Arenal, F. 2014. Chapter Three - Evolution and Emergence of Plant Viruses. In Advances in Virus Research, eds. Karl Maramorosch and Frederick A. Murphy. Academic Press, p. 161–191.

Folmer, O., Black, M., Wr, H., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology. 3:294–9.

Fontdevila Pareta, N., Khalili, M., Maachi, A., Rivarez, M. P. S., Rollin, J., Salavert, F., et al. 2023. Managing the deluge of newly discovered plant viruses and viroids: an optimized scientific and regulatory framework for their characterization and risk analysis. Frontiers in Microbiology. 14.

Fox, A. 2020. Reconsidering causal association in plant virology. Plant Pathology. 69:956–961.

Fránová, J., Lenz, O., Přibylová, J., Čmejla, R., Valentová, L., and Koloniuk, I. 2021. High Incidence of Strawberry Polerovirus 1 in the Czech Republic and Its Vectors, Genetic Variability and Recombination. Viruses. 13:2487.

Gaafar, Y. Z. A., Abdelgalil, M. a. M., Knierim, D., Richert-Pöggeler, K. R., Menzel, W., Winter, S., et al. 2017. First Report of physostegia chlorotic mottle virus on Tomato (Solanum lycopersicum) in Germany. Plant Disease. 102:255–255.

Giustina, W. D., Javoy, M., Bansept, P., Morel, E., Balasse, H., Goussard, N., et al. 2000. Les cicadelles du genre Anaceratagallia vectrices du virus responsable de la maladie de la peau de crapaud du concombre. PHM Revue Horticole. :40.

Hanssen, I. M., and Thomma, B. P. H. J. 2010. Pepino mosaic virus: a successful pathogen that rapidly evolved from emerging to endemic in tomato crops. Molecular Plant Pathology. 11:179–189.

Hogenhout, S. A., Redinbaugh, M. G., and Ammar, E. D. 2003. Plant and animal rhabdovirus host range: a bug's view. Trends in microbiology.

Hou, W., Li, S., and Massart, S. 2020. Is There a "Biological Desert" With the Discovery of New Plant Viruses? A Retrospective Analysis for New Fruit Tree Viruses. Front. Microbiol. 11.

Hull, R., ed. 2014. Front-matter. In Plant Virology (Fifth Edition), Boston: Academic Press, p. i–iii.

Jones, R. A. C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Research. 100:5–30.

Jones, R. A. C. 2009. Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Research. 141:113–130.

Jones, R. A. C., and Naidu, R. A. 2019. Global Dimensions of Plant Virus Diseases: Current Status and Future Perspectives. Annual Review of Virology. 6:387–409.

Lefeuvre, P., Martin, D. P., Elena, S. F., Shepherd, D. N., Roumagnac, P., and Varsani, A. 2019. Evolution and ecology of plant viruses. Nature Reviews Microbiology. 17:632–644.

Levy, D., and Lapidot, M. 2008. Effect of plant age at inoculation on expression of genetic resistance to tomato yellow leaf curl virus. Arch Virol. 153:171–179.

Liefting, L. W., Waite, D. W., and Thompson, J. R. 2021. Application of Oxford Nanopore Technology to Plant Virus Detection. Viruses. 13:1424.

Massart, S., Candresse, T., Gil, J., Lacomme, C., Predajna, L., Ravnikar, M., et al. 2017. A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies. Front. Microbiol. 8.

Meleshko, D., Hajirasouliha, I., and Korobeynikov, A. 2021. coronaSPAdes: from biosynthetic gene clusters to RNA viral assemblies. Bioinformatics. 38:1–8.

Menzel, W., Richert-Pöggeler, K., Winter, S., and Knierim, D. 2018. Characterization of a nucleorhabdovirus from Physostegia. Acta Horticulturae. 1193:29–38.

Nickel H, Remane R, 2002. Check list of the planthoppers and leafhoppers of Germany, with notes on food plants, diet width, life cycles, geographic range and conservation status (Hemiptera, Fulgoromorpha and Cicadomorpha). Beiträge zur Zikadenkunde 5.

Oladokun, J. O., Halabi, M. H., Barua, P., and Nath, P. D. 2019. Tomato brown rugose fruit disease: current distribution, knowledge and future prospects. Plant Pathology. 68:1579–1586.

Reynard, J.-S., Brodard, J., Zufferey, V., Rienth, M., Gugerli, P., Schumpp, O., et al. 2022. Nuances of Responses to Two Sources of Grapevine Leafroll Disease on Pinot Noir Grown in the Field for 17 Years. Viruses. 14:1333.

Rivarez, M. P. S., Vučurović, A., Mehle, N., Ravnikar, M., and Kutnjak, D. 2021. Global Advances in Tomato Virome Research: Current Status and the Impact of High-Throughput Sequencing. Frontiers in Microbiology. 12.

Roggero, P., Milne, R. G., Masenga, V., Ogliara, P., and Stravato, V. M. 1995. First reports of eggplant mottled dwarf rhabdovirus in cucumber and in pepper. Plant Disease. 79.

S, C., Y, Z., Y, C., and J, G. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics (Oxford, England). 34.

Sf, A., W, G., W, M., Ew, M., and Dj, L. 1990. Basic local alignment search tool. Journal of molecular biology. 215.

Spence, N. J., Basham, J., Mumford, R. A., Hayman, G., Edmondson, R., and Jones, D. R. 2006. Effect of Pepino mosaic virus on the yield and quality of glasshouse-grown tomatoes in the UK. Plant Pathology. 55:595–606.

Spence, N. J., Phiri, N. A., Hughes, S. L., Mwaniki, A., Simons, S., Oduor, G., et al. 2007. Economic impact of Turnip mosaic virus, Cauliflower mosaic virus and Beet mosaic virus in three Kenyan vegetables. Plant Pathology. 56:317–323.

Tamburini, G., Bommarco, R., Wanger, T. C., Kremen, C., van der Heijden, M. G. A., Liebman, M., et al. 2020. Agricultural diversification promotes multiple ecosystem services without compromising yield. Science Advances. 6:eaba1715.

Temple, C., Blouin, A. G., De Jonghe, K., Foucart, Y., Botermans, M., Westenberg, M., et al. 2022. Biological and Genetic Characterization of Physostegia Chlorotic Mottle Virus in Europe Based on Host Range, Location, and Time. Plant Disease. 106:2797–2807.

Temple, C., Blouin, A. G., Tindale, S., Steyer, S., Marechal, K., and Massart., S. 2023. High Throughput Sequencing technologies complemented by growers' perceptions highlight the impact of tomato virome in diversified vegetable farms and a lack of awareness of emerging virus threats. 2023. Front. Sustain. Food Syst. Sec. Crop Biology and Sustainability. 7.

Tishechkin. 2020. ‘Review of the Leafhopper Genus Anaceratagallia Zachvatkin, 1946 (Homoptera: Auchenorrhyncha: Cicadellidae: Megophthalminae: Agalliini) from Russia, Kazakhstan, and Central Asia’. Zootaxa 4821 (2): 250–76.

Trebicki, P. 2020. Climate change and plant virus epidemiology. Virus Research. 286:198059.

Vučurović, A., Kutnjak, D., Mehle, N., Stanković, I., Pecman, A., Bulajić, A., et al. 2021. Detection of Four New Tomato Viruses in Serbia Using Post Hoc High-Throughput Sequencing Analysis of Samples From a Large-Scale Field Survey. Plant Disease. 105:2325–2332.

Whitfield, A. E., Huot, O. B., Martin, K. M., Kondo, H., and Dietzgen, R. G. 2018. Plant rhabdoviruses—their origins and vector interactions. Current Opinion in Virology. 33:198–207.

Chapter 5

Discussion

This thesis first aimed to better understand the issues associated with viral diseases in tomato plants within Belgian diversified production systems. From this, the work has been further focused on the biological characterization of a newly emergent disease present at a European level and caused by physostegia chlorotic mottle virus (PhCMoV).

1. Contribution to the development of an interdisciplinary approach in plant virus research.

While the focus of this work was primarily based on phytopathological investigations, efforts were made to integrate socio-economic aspects in a first survey to have a more holistic and contextualized overview of the problems, as recommanded by Deguine et al., (2023). To start, the tomato virome was studied in Belgian farms with an interdisciplinary approach combining growers' perception and production systems characterization with symptom observations and HTS identification of viruses in the field. The objective of this qualitative investigation was double: to explore and gain insight into tomato virus-related issues in a specific production system (diversified production for fresh market), and to formulate hypotheses about the factors that could contribute to these problems (cultural practices, perception…).

The main findings indicated that while some viruses associated with tomato yield losses were present in the fields, most growers did not experience major problems related to plant viruses, and the number of symptomatic plants was low. Nevertheless, it allowed to i) assess the virome in Belgium's diversified production systems as it is recommended for plant biosecurity by MacDiarmid et al., 2013, ii) to communicate with growers about the main threat related to tomato viruses in Belgium (ToBRFV) and the threatening viruses detected in their field (ToMV, PhCMoV) and iii) to identify a new plant virus that was needed biological characterization (PhCMoV). Overall, the fact that PhCMoV was problematic in two farms out of nine where it was present highlights that continuing to study plant viruses in diversified production systems is essential to sustainably avoid outbreaks.

Exchanging with producers does not seem to be a common practice in research on viral diseases of tomatoes, or at least, it is not explicitly stated in the papers since the development of HTS usage for tomato virus studies (Xu et al., 2017, Desbiez et al., 2020, Li et al., 2021, Rivarez et al., 2023). However, since cultural practices and human actions can influence the spread of viruses, it can be essential to study cultural practices to understand the epidemiology of plant viruses (e.g. cassava viruses: Nyirakanani et al., 2021, Szyniszewska et al., 2021, Kwibuka et al., 2022).

Furthermore, from a philosophical perspective, it is also relevant to be in touch with producers and to communicate with them about plant viruses (at least to communicate about the results) while studying them, as they are the first to be affected by these issues. In many different scientific fields, the fragmentation and specialization of research have led to a growing disconnection from real-world society (Hatt et al., 2016). Still, to foster a sustainable transition, it is recommended to incorporate greater

trans-disciplinarity in the implementation of research projects (Wezel et al., 2014, Hatt et al., 2016, Deguine et al., 2023).

In this thesis, exchanging information with producers was beneficial in efficiently understanding the risks associated with plant viruses and better understanding the need of growers. Moreover, producers have also derived benefits from this collaborative approach. Feedback was given to the producers and walloon extension services (Interprofessional Center of vegetable growers, (CIM)) on what could drive the spread of viruses identified (i.e. by providing information on the pathogen life cycle and useful website links). During the biological characterization of PhCMoV, producers that encountered difficulties and the CIM were regularly informed of the research's progress.

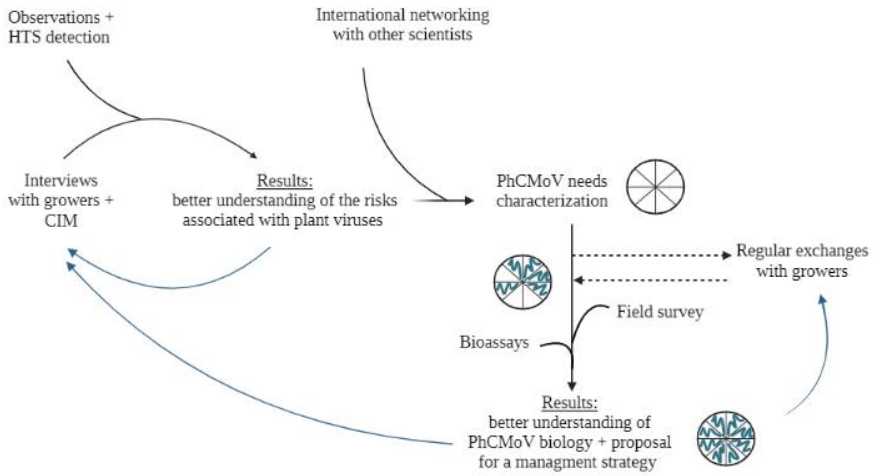


Figure 5-1. Logical pathway of my PhD thesis, including feedback to the growers (blue arrows). The pie chart represents the last pie chart of the framework described in Figure 1-7. After the first study on PhCMoV, aspects related to its genetic diversity, host range, symptomatology and geographical distribution were filled. Then, the second study allowed to fill the missing knowledge gaps on the transmission, severity and incidence of PhCMoV and to complete its characterization.

Understanding virus avoidance cultural practices was one of the primary reasons why producers in Belgium were interested in participating in the survey, especially given that chemical control is often not an option in most studied production systems. In this thesis, it was observed that growing perennial plants in a tunnel close to annual plants (e.g., tomato, cucumber) for an extended period could increase risks of PhCMoV prevalence. While further research is necessary to confirm these findings, they appear to have been beneficial for one grower who opted to remove the perennial plants from their annual crop tunnels. This action led to a significant reduction in PhCMoV-related problems in the same location the year after. Having informed this

producer on the biology of the virus throughout the research appeared to be beneficial, as she reported, "The list of host plants sent was very helpful, I paid close attention to the mallow when weeding the tunnel!". However, it is challenging to attribute the decrease in issues due to PhCMoV entirely to this action (removing the plants inside the tunnel) as many other environmental factors could have played a role. Conducting a long-term study in collaboration with multiple growers can be valuable in validating this statement.

Overall, improving communication and connections with producers alongside plant viral research needs to be considered in future research projects on plant viral diseases for practical and philosophical reasons. The approach used in this thesis could be adapted and applied to many other contexts where plant viral diseases are studied in cultivated area. The methodology could be simplified to be used more systematically and on a larger scale in field virus studies (e.g. small questionnaire of 5-15 minutes only).

Difficulties encounter:

During this qualitative survey, we attempted to assess the risks associated with viruses while seeking to understand them. Therefore, it was challenging to identify robust socio-economical or agronomical factors explaining of the presence and impact of viruses or their potential associated-risks. In addition, the problems associated with plant viral diseases were relatively minor, which also challenged to understand explanatory socio-ecological and agronomical factors. To better incorporate socio-economic elements into studying plant diseases, it could be relevant to comprehensively understand the potential issues in the studied context and defining precise research objectives beforehand. Thereafter, the questions that need to be addressed must require an integrated approach recognizing the limitations inherent in the compartmentalized nature of academic research (Mendez et al., 2013; Hatt et al., 2016). In the frame of this research, a collaboration with socio-economic researchers to design the methodology and analyze the data was initiated. Nevertheless, plant virology differs significantly from socio-economic sciences, and more time, regular communication, and training could have been beneficial in better integrating these two disciplines. Integrating these disciplines with natural sciences requires an open-minded approach and significant effort that should not be underestimated (Mendez et al., 2013; Hatt et al., 2016, Kelly et al., 2019).

Further prospect:

Despite the absence of ToBRFV detection in the survey, the fact that some growers acknowledged their lack of awareness regarding ToBRFV suggested that understanding how they can access information about plant viral diseases and exploring communication dynamics between regulatory agencies and alternative growers could have been an important avenue for further understanding the risks associated with plant viral diseases in Belgium. This study found that not all growers registered at extension services (CIM) were aware of ToBRFV (while they sent alerts)

and not only those who were aware were followed by the extension services. This highlights that access to information about quarantine plant diseases is not as straightforward as assumed, and there may be gaps in the communication between the extension services and the growers. During the interviews conducted for this study, information about ToBRFV was shared with the growers. It is urgent to communicate more widely to small-scale producers about this virus to avoid outbreaks in these production systems because ToBRFV is highly contagious, and any producer may likely encounter it at some point. ToBRFV can contaminate the food chain and ecosystems and persist in the environment for extended periods (Klap et al., 2020, Zhang et al., 2022). Primary introduction of the virus in a production system is therefore very likely because it can occur through tools, water, seeds, infected fruits from the supermarket and compost, bumblebees, seeds, tomato containers, workers etc. (Bačnik et al., 2020, Klap et al., 2020, Zhang et al., 2022). In addition, in Belgium and the Netherlands, illegal cross-protection of ToBRFV was performed by some growers, increasing the prevalence of the virus in the environment and, thus, the risk of spread. Developing management strategies to control ToBRFV is essential in reducing its prevalence in the environment and minimizing the risk of spreading to diversified production systems. This is particularly important because eliminating the virus from such systems would be highly challenging.

2. Focus on PhCMoV

For the second axis of this thesis, the biological characterization of PhCMoV was initiated to evaluate its associated risks for the production following the frameworks described by Massart et al., 2017 and recently improved by Fontdevila et al., 2023.

During this thesis, maximizing exchanges with experts from various field (in or outside academia) and surveying the disease in the field was highly valuable in improving its biological characterization. Overall, this thesis was a good example of how biological characterization of a new virus can be optimized by collaboration with multiple stakeholders and testify that it can be better considered in the future, especially regarding the pace of discovery of new plant viruses and the need to better contextualized plant virus studies alongside growers realities (He et al., 2016, Hou et al., 2019, Rivarez et al., 2021).

The initial collaboration with eight research groups working on plant viruses from five European countries was valuable in creating synergies and avoiding redundancies in setting up the experiments. This enabled to set up trials in laboratories where the infrastructures were best suited to carry them out, to exchange infected material, antibodies, tips and protocols to optimize trials. The cooperation has enabled a wider perspective on the disease and consolidated the most comprehensive information in a single publication, instead of eight new disease reports from individual countries. As a result, the virus was characterized more quickly and comprehensively, and the scope of its host range and infection dynamics across different European countries were

better understood. A list of virus isolates was also compiled, facilitating the study of its diversity and phylogeny.

Subsequently, to address biological knowledge gaps identified in the first study, experiments were developed based on the knowledge previously acquired and the literature, frequent visit in the field, and on insights from experienced colleagues, producers, and other stakeholders from diverse backgrounds (entomologists, horticultural school…). For example, the identification of the vector was accomplished based on the comparison of field observations with what was known in the literature (e.g. transmission of alphanucleorhaboviruses by leafhoppers belonging to the *Anaceratagalliae* genus, Della Giustina et al., 2000, Dietzgen et al., 2020, Dietzgen et al., 2021).

In addition, studying the disease in its natural environment was advantageous and saved a lot of time. Bioassays were designed to better understand the disease after observation in the field. For example, the association between the presence of the virus and severe symptoms on tomato fruits was demonstrated in the laboratory after the detection of the virus on multiple symptomatic different tomato cultivars (Figure 5-2). This would not have been possible without the agreement of growers. In this study, they were keen to help (which may not always be the case). They allowed repeated access to their fields for plant collection, including perennial plants with roots. They offered to delay the removal of infected plants for a detailed study and to grow sentinels plants to monitor the virus's presence. Additionally, some producers shared their assumptions and reflections about the disease, which was helpful and sometimes confirmed what was observed in experimental conditions.

Figure 5-2. PhCMoV infected tomato plants in the field

Regarding further experiments, this research and the literature on plant rhabdoviruses suggest that the ecology and behaviour of PhCMoV's vector (*Anaceratagallia* sp.) is a crucial next step to determine if the impact of PhCMoV will increase and how to manage it (Hogenhout et al., 2008, Dietzgen et al., 2020, Whitfield et al., 2018).

One of the first tests is to identify the vector of PhCMoV at a species level, and to evaluate if other *Anaceratagallia* species (such as *A. fragariae*) can transmit the virus. From a larger perspective, assessing whether transmission of PhCMoV and EMDV is specific to insect species or genera and studying the prevalence of these insect species in Europe may help to better understand the distribution of these two rhabdoviruses.

Thereafter it would be crucial to simultaneously understand the ecology and biology of the PhCMoV vectors (i.e. their geographical distribution, on which plants they reproduce, under which conditions etc.) and their interactions with the virus (i.e. acquisition, latency, inoculation and retention time, transmission to the progeny, impact on the host fitness and behaviour). Overall, it can be noticed that the distribution of *Anaceratagallia* species is mainly located in Europe, such as the distribution of PhCMoV (Figure 5-3).

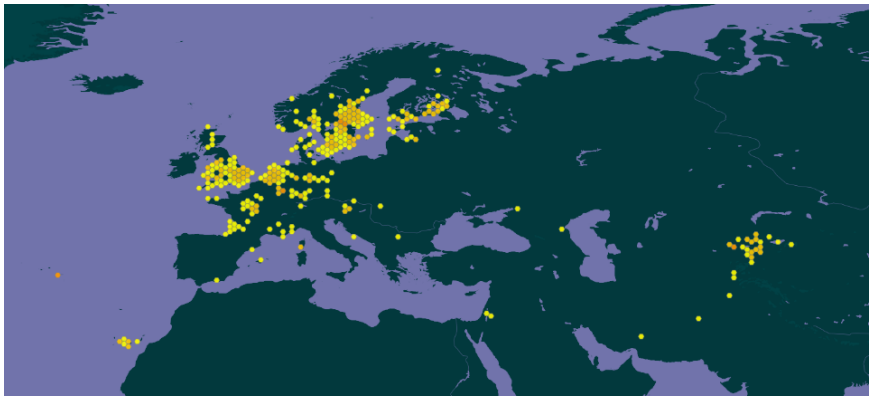


Figure 5-3: Georeferenced records (1851 - 2023) of leafhoppers from the genera *Anaceratagalliae* in the EU. Orange dots stand for a higher insect density (Global Biodiversity Information Facility, 2021)

The genus *Anaceratagalliae* has been recently revised and the authors suggested that there is much confusion with identification of the species within this genus (Tishechkin, 2020). Indeed, Tishechkin, 2020 suggested that previous entomologists overestimated the significance of minor differences in the shape of male apodemes and genitalia and as a result, described intraspecific variants as new species. Some taxa were also described on a single male, which did not allow the investigation of intraspecific variability. Tishechkin, 2020 reclassified the species of *Anaceratagalliae*

genus into four species groups: *A. laevis*, *A. ribauti*, *A. venosa*, and *A. acuteangulata* based on the shape of male genitalia.

Phylogenetic analysis based on *Anaceratagallia* COI available in the NCBI database and the specimens that were collected in Belgium (including some which transmit PhCMoV, Figure 5-4) showed three distinct species groups: *A ribauti*, *A. venosa* and an unnamed group of species which could therefore be part of the subgroup *A. laevis* or *A. acuteangulata*.

In a transmission assay, Giustina et al., (2000) demonstrated that the transmission of "an EMDV strain" was more efficient for *A. laevis* than *A. ribauti*. Nevertheless, since the diagnosis for the virus was only based on symptoms observation, this is questioning whether Giustina et al., 2000 could have investigated PhCMoV instead of EMDV. If by mistake they were working with PhCMoV because the symptoms are identical to those of EMDV and we now know that PhCMoV coexists in this region, then *A. laevis* or a species belonging to this species group could be the best candidate for the unnamed *Anaceratagallia* species which can transmit PhCMoV. Nevertheless, this remains to be verified.

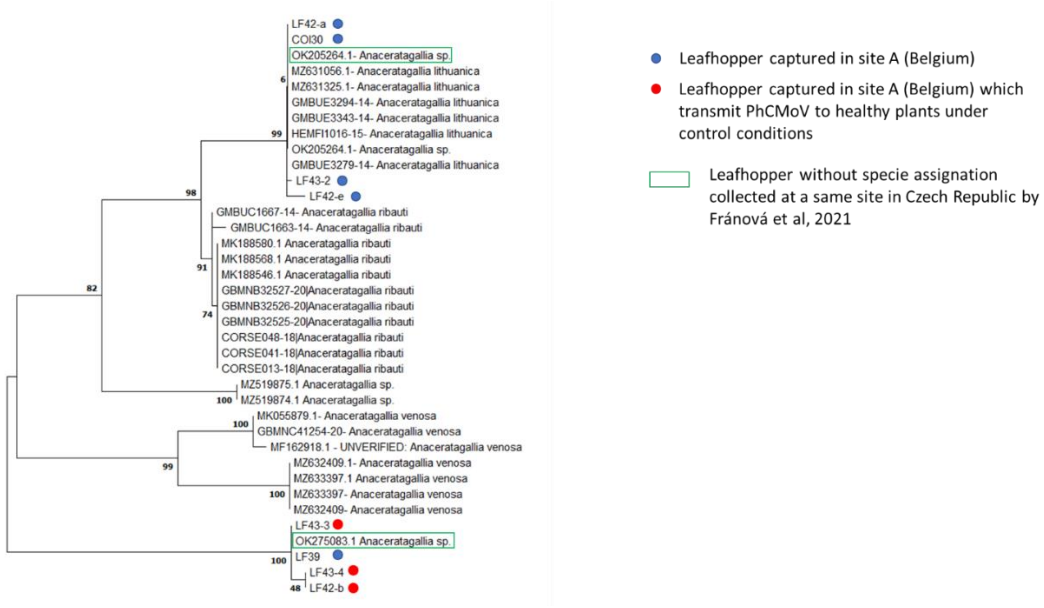


Figure 5-4: Maximum Likelihood evolutionary analysis of published *Anaceratagallia* COI nucleotidique sequences and *Anaceratagallia* captured in PhCMoV infected belgium site

Further collaboration with entomologists is necessary to conduct such a study, and sufficient time should be allocated since rearing and manipulating leafhoppers is challenging and requires expertise.

The host range of PhCMoV seems to be extensive and currently not fully caracterized, possibly due to the polyphagous behavior of its vector, *Anaceratagallia*.

The research findings indicate that controlling the damage caused by the virus could be achieved by removing perennial plants in tomato tunnels. These plants can act as reservoirs for the virus and its vector. However, growers might hesitate to take this action. In addition, if PhCMoV-related issues are present in unprotected crops or tunnels without reservoir plants, it will be challenging to recommend the removal of perennial plants in the entire site because the virus was mainly identified in production systems based on the cultivation of a high diversity of plants.

Nonetheless, gaining knowledge about the primary plants that can host the virus during winter, their symptomatic expression when infected, and the plants that support reproduction and overwintering of insect vectors would allow for more targeted and tailored proposals for plant removal in these production systems, thereby improving disease management strategies. This thesis identifies sorrel as a strong candidate for removal due to its suitability for *Anaceratagallia* reproduction and its ability to host the virus. Additionally, mallow could be further studied for its ability to host the vector because several infected mallow plants have been found near an annual plant tunnel that remains infected with the virus for over four consecutive years. Conducting surveys and tests on the cultivated perennial plants in PhCMoV-infected plots may help assess their potential as hosts for the virus and its vector.

Another management strategy that can potentially be developed is to safeguard the sensitive plants from being in contact with the leafhoppers at the beginning of the season when the infection can be more impactful for the production by protecting all entrances of the tunnels with leafhoppers-proof netting (Jones et al., 2004).

In this thesis, communication about PhCMoV research was continuously done with the producers with whom we were in contact and with extension services and scientists (through fact sheets or publication, Figure 5-1). Nevertheless, more communication on the disease can be achieved with extension services and growers association in Flanders, the Netherlands, France and other European countries where the disease is present.

Overall, even if the characterization of this novel viral species was more efficient than previously possible and envisioned (due to the HTS technological advancements and trust between scientists, technicians and growers), this was still a long process involving many resources. The question arises whether adequate resources will be available to conduct similar extensive biological studies on emerging viruses in the future. This is especially concerning for plant viruses like PhCMoV, which may impact vegetables grown in understudied small-scale production systems that receive less support from governments or funding agencies than industrial production systems.

As an alternative, innovative prediction tools based on machine learning approaches on protein features may be further developed and used to predict the biological characteristics and, thus, risks associated with a new or poorly understood virus (eg. how it may be transmitted, to which host plants, survival strategies of a virus, aggressiveness…) (Tahzima et al., 2021, Fontdevila et al., 2023). However, supposing that these tools can efficiently predict key biological aspects of plant

viruses and that biological experimentations will not be needed anymore, enhancing communication with growers and other stakeholders will likely be even more crucial to build trust in the results.

When starting this work, knowledge of PhCMoV was limited to its ability to cause disease on tomato and physostegia in Austria and Germany. With the connection of multiple stakeholders, PhCMoV is now one of the best-characterized new tomato viruses after ToBRFV (Figure 5-3).

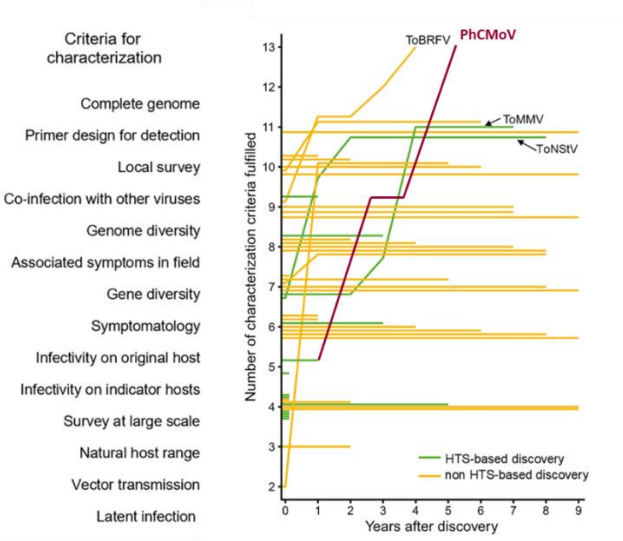


Figure 5-3. Post-discovery characterization of new tomato-infecting viruses according to the literature review (adapted from Rivarez et al., 2021)

Although the virus can result in significant tomato yield losses, it is currently not very efficiently transmitted from plant to plant. However, this study suggests that if the disease is present on a site, growing perennial plants, such as sorrel, which can host both the virus and its vector within tunnels where tomatoes are also grown, may increase the risk of spreading the disease to young plants and lead to yield losses.

This phenomenon might be the case for other plant viruses such as melon chlorotic spot virus, a virus which may also deserve further biological characterization as it has recently started to be detected in symptomatic plants (e.g. Annexe 1, data not shown). Overall, this thesis suggests that a subtle understanding of the interactions between plants, diseases, insects and cultural practices in diversified production systems and increased connections with producers and other stakeholders can enable to manage viral disease efficiently and sustainably.

Personnal note

Throughout my PhD, I often questioned the role of plant virology research in the urgent need for a transition to a more sustainable agricultural production model, especially in the current global context where plant viruses can still seriously impact food security. Given the current ecological and social crisis, it seems essential to question and reflect on the impact of our research on the real world in a broad and distant context (over several years) (Hatt et al., 2016). This motivated me to shift my attention towards small-scale producers difficulties regarding plant viruses and to connect with them.

From my perspective, it would be interesting to discuss these issues during peer conferences and then in debates with farmers/citizens to better understand our roles and how to position ourselves, particularly as young researchers in the current context. This would also help the general public and researchers to understand each other better, which could be beneficial for promoting inter and transdisciplinary research. It is also crucial that funding agencies support such initiatives for a better agricultural transition.

From what I experienced in this PhD, it is not easy to undertake inter/trans-disciplinary studies. Still, little initiatives can be helpful to improve the link between society and researchers and be a starting point for building synergies between plant health stakeholders.

References

Bačnik, K., Kutnjak, D., Pecman, A., Mehle, N., Tušek Žnidarič, M., Gutiérrez Aguirre, I., et al. 2020. Viromics and infectivity analysis reveal the release of infective plant viruses from wastewater into the environment. Water Res. 177:115628.

Baker, M. B., Venugopal, P. D., and Lamp, W. O. 2015. Climate Change and Phenology: Empoasca fabae (Hemiptera: Cicadellidae) Migration and Severity of Impact. PLOS ONE. 10:e0124915.

Deguine, J.-P., Aubertot, J.-N., Bellon, S., Côte, F., Lauri, P.-E., Lescourret, F., et al. 2023. Agroecological crop protection for sustainable agriculture. In *Advances in Agronomy*, Academic Press.

Desbiez, C., Wipf-Scheibel, C., Millot, P., Berthier, K., Girardot, G., Gognalons, P., et al. 2020. Distribution and evolution of the major viruses infecting cucurbitaceous and solanaceous crops in the French Mediterranean area. Virus Research. 286:198042.

Dietzgen, R. G., Bejerman, N. E., Goodin, M. M., Higgins, C. M., Huot, O. B., Kondo, H., et al. 2020. Diversity and epidemiology of plant rhabdoviruses. Virus Research. :197942.

Dietzgen, R. G., Bejerman, N. E., Mei, Y., Jee, C. L. J., Chabi-Jesus, C., Freitas-Astúa, J., et al. 2021. Joá yellow blotch-associated virus, a new alphanucleorhabdovirus from a wild solanaceous plant in Brazil. Arch Virol.

Giustina, W. D., Javoy, M., Bansept, P., Morel, E., Balasse, H., Goussard, N., et al. 2000. Les cicadelles du genre Anaceratagallia vectrices du virus responsable de la maladie de la peau de crapaud du concombre. PHM Revue Horticole. :40.

Hatt*, S., Artru*, S., Brédart, D., Lassois, L., Francis, F., Haubruge, É., et al. 2016. Towards sustainable food systems: the concept of agroecology and how it questions current research practices. A review. Biotechnol. Agron. Soc. Environ.

Hogenhout, S. A., Ammar, E.-D., Whitfield, A. E., and Redinbaugh, M. G. 2008. Insect Vector Interactions with Persistently Transmitted Viruses. Annual Review of Phytopathology. 46:327–359.

Hou, W., Li, S., and Massart, S. 2020. Is There a "Biological Desert" With the Discovery of New Plant Viruses? A Retrospective Analysis for New Fruit Tree Viruses. Front. Microbiol. 11.

Jones, R. A. C. 2009. Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Research. 141:113–130.

Jones, R. A. C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Research. 100:5–30.

Kelly, R., Mackay, M., Nash, K. L., Cvitanovic, C., Allison, E. H., Armitage, D., et al. 2019. Ten tips for developing interdisciplinary socio-ecological researchers. Socio Ecol Pract Res. 1:149–161.

Klap, C., Luria, N., Smith, E., Bakelman, E., Belausov, E., Laskar, O., et al. 2020. The Potential Risk of Plant-Virus Disease Initiation by Infected Tomatoes. Plants. 9:623.

Kwibuka, Y., Nyirakanani, C., Bizimana, J. P., Bisimwa, E., Brostaux, Y., Lassois, L., et al. 2022. Risk factors associated with cassava brown streak disease dissemination through seed pathways in Eastern D.R. Congo. Frontiers in Plant Science. 13.

Li, Y., Tan, G., Xiao, L., Zhou, W., Lan, P., Chen, X., et al. 2021. A Multiyear Survey and Identification of Pepper- and Tomato-Infecting Viruses in Yunnan Province, China. Frontiers in Microbiology. 12.

MacDiarmid, R., Rodoni, B., Melcher, U., Ochoa-Corona, F., and Roossinck, M. 2013. Biosecurity Implications of New Technology and Discovery in Plant Virus Research. PLOS Pathogens. 9:e1003337.

Massart, S., Candresse, T., Gil, J., Lacomme, C., Predajna, L., Ravnikar, M., et al. 2017. A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies. Front. Microbiol. 8.

E., Bacon, C. M., and Cohen, R. 2013. Agroecology as a Transdisciplinary, Participatory, and Action-Oriented Approach. Agroecology and Sustainable Food Systems. 37:3–18.

Nyirakanani, C., Bizimana, J. P., Kwibuka, Y., Nduwumuremyi, A., Bigirimana, V. de P., Bucagu, C., et al. 2021. Farmer and Field Survey in Cassava-Growing Districts of Rwanda Reveals Key Factors Associated With Cassava Brown Streak Disease Incidence and Cassava Productivity. Frontiers in Sustainable Food Systems. 5.

Rivarez, M. P. S., Pecman, A., Bačnik, K., Maksimović, O., Vučurović, A., Seljak, G., et al. 2023. In-depth study of tomato and weed viromes reveals undiscovered plant virus diversity in an agroecosystem. Microbiome. 11:60.

Rivarez, M. P. S., Vučurović, A., Mehle, N., Ravnikar, M., and Kutnjak, D. 2021. Global Advances in Tomato Virome Research: Current Status and the Impact of High-Throughput Sequencing. Front. Microbiol. 12.

Szyniszewska, A. M., Chikoti, P. C., Tembo, M., Mulenga, R., Gilligan, C. A., van den Bosch, F., et al. 2021. Smallholder Cassava Planting Material Movement and Grower Behavior in Zambia: Implications for the Management of Cassava Virus Diseases. Phytopathology®. 111:1952–1962.

Tahzima, R., Haegeman, A., Massart, S., and Hébrard, E. 2021. Chapter Ten - Flexible spandrels of the global plant virome: Proteomic-wide evolutionary patterns of structural intrinsic protein disorder elucidate modulation at the functional virus–host interplay. In *Progress in Molecular Biology and Translational Science*, Dancing Protein Clouds: Intrinsically Disordered Proteins in the Norm and Pathology, Part C, ed. Vladimir N. Uversky. Academic Press, p. 355–409.

Tishechkin, D. Y. 2020. Review of the leafhopper genus Anaceratagallia Zachvatkin, 1946 (Homoptera: Auchenorrhyncha: Cicadellidae: Megophthalminae: Agalliini) from Russia, Kazakhstan, and Central Asia. Zootaxa. 4821:250–276.

Wezel, A., Casagrande, M., Celette, F., Vian, J.-F., Ferrer, A., and Peigné, J. 2014. Agroecological practices for sustainable agriculture. A review. Agronomy for Sustainable Development. 34:1–20.

Whitfield, A. E., Huot, O. B., Martin, K. M., Kondo, H., and Dietzgen, R. G. 2018. Plant rhabdoviruses—their origins and vector interactions. Current Opinion in Virology. 33:198–207.

Xu, C., Sun, X., Taylor, A., Jiao, C., Xu, Y., Cai, X., et al. 2017. Diversity, Distribution, and Evolution of Tomato Viruses in China Uncovered by Small RNA Sequencing. J. Virol. 91.

Zhang, S., Griffiths, J. S., Marchand, G., Bernards, M. A., and Wang, A. Tomato brown rugose fruit virus: An emerging and rapidly spreading plant RNA virus that threatens tomato production worldwide. Molecular Plant Pathology.

ANNEXE 1: Disease note: First report of Melon chlorotic spot virus in Belgium and in cultivated sorrel (Rumex acetosa) (submitted to Plant disease)

Temple, C., Blouin A.G., Steyer, S., Massart S.

In 2020, symptoms of putative viral origin were observed on 7% of tomatoes in an organic vegetable farm in Belgium (deformed uneven ripened fruits, vein clearing, mosaic and purple leaves, stunted plants). The leaves of twenty symptomatic plants were collected, pooled and screened for viruses using high throughput sequencing technologies (HTS) on Illumina NextSeq500 following a virion-associated nucleic acid (VANA) protocol described previously (Temple et al., 2021, sample Be_SL1). In total, 3665498 (PE150) reads were generated and bioinformatic analyses (denovo assembly, tblastx search on updated NCBI database and mapping on the closest reference) using Geneious Prime® 2020.1.2 revealed the presence of three viruses known to infect tomatoes: physostegia chlorotic mottle virus (PhCMoV, 547,142 reads map on NC_055466, potato virus Y (PVY, 4056 reads map on MW595184), and melon chlorotic spot virus (MeCSV, 55 reads mapped to six out of the eight different MeCSV segments (NC_040448-55). PhCMoV and MeCSV belong, respectively to *Alphanucleorhabdovirus* and *Tenuivirus* genus. The high level of prevalence triggered the research of alternative perennial hosts that can serve as a reservoir during inter-cropping season. One plant of *Rumex acetosa* showing vein clearing (CT-122) was collected in the same greenhouse the year after. Total RNA was extracted, followed by ribodepletion, and Illumina HTS using the protocol described in Temple et al., (2021) for sample Be_GP1. In total, 4,707,544 PE150 reads were obtained and bioinformatic analyses confirmed the presence of MeCSV (4727 reads mapped on eight RNA segments NC_040448-55) and suggested the presence of an unclassified partitivirus (1652 reads mapped on NC_040457 with 11.9% of ref seq). RNA1 segment was used to design MeCSV-specific RT-PCR primers for detection (MeCSV-125F 5'-TTTAAGGCCAGATCCAGAGGTTC-3'/ MeCSV-498R 5'-TGGATGTGACAACCTGGTAGTAC-3').

Thereafter, in July 2022, 42 *R. acetosa* plants were collected in the same greenhouse. Among them, seven plants showed vein clearing, two showed yellowing with necrosis, two exhibited yellowing and vein clearing, and one showed mosaic. The other 30 plants did not show any apparent symptoms. The 42 plants were subjected to RNA extraction and RT-PCR for MeCSV and PhCMoV detection. MeCSV was detected in 13 plants (all the symptomatic plants except the one exhibiting mosaic where PhCMoV was detected, and two asymptomatic plants). PhCMoV was also detected in three plants with vein clearing, one with yellowing and one of the two asymptomatic plants infected by MeCSV.

Our results report the first detection of MeCSV in *R. acetosa*. This is also the first detection of MeCSV in Belgium. In addition, according to the hierarchical approach for assessing causal relationships in plant virology (Fox et al., 2020), a preliminary association was observed between symptoms and MeCSV detection [6% prevalence on healthy plants and 92% prevalence on diseased plants (from which seven symptomatic samples were not co-infected by PhCMoV)]. Symptom causality should be further investigated but our results are important for disease management because they suggested that cultivated perennial *R. acetosa* may serve as a reservoir for two emergent plant viruses (PhCMoV and MeCSV), associated with symptoms on tomato (Lecoq et al., 2019, Temple et al., 2021).

References

Fox, A. 2020. Reconsidering causal association in plant virology. Plant Pathology. 69:956–961.

Lecoq, H., Wipf-Scheibel, C., Verdin, E., and Desbiez, C. 2019. Characterization of the first tenuivirus naturally infecting dicotyledonous plants. Arch Virol. 164:297–301.

Temple, C., Blouin, A. G., De Jonghe, K., Foucart, Y., Botermans, M., Westenberg, M., et al. 2022. Biological and Genetic Characterization of Physostegia Chlorotic Mottle Virus in Europe Based on Host Range, Location, and Time. Plant Disease. 106:2797–2807.

Acknowledgments

This work was supported by the European Union's Horizon 2020 Research and Innovation Program under the Marie SkłodowskaCurie, grant agreement nos. 813542 and 871029 and Federal Public Service, Public Health, Belgium, grant agreement no. RT 18/3 SEVIPLANT.

Publications and conferences

Publications

Temple, C., Blouin, A.G., De Jonghe, K., Foucart, Y., Botermans, M., Westenberg, M., Schoen, R., Gentit, et al., 2022. Biological and Genetic Characterization of Physostegia Chlorotic Mottle Virus in Europe Based on Host Range, Location, and Time. Plant Disease 106, 2797–2807.

Temple, C., Blouin, A.G., Tindale, S., Steyer, S., Marechal, K., Massart, S., 2023. High Throughput Sequencing technologies complemented by grower's perception highlight the impact of tomato virome in diversified vegetable farms and a lack of awareness of emerging virus threats. Front. Sustain. Food Syst. Sec. Crop Biology and Sustainability. Volume 7.

Temple, C., Blouin, A.G., Boezen D., Botermans, M., Durant, L., De Jonghe K., De Koning, P., Goedefroit T., Minet L., Steyer, S., Verdin, E., Zwart, M., Massart, S., Biological characterization of an emergent virus infecting vegetables in diversified production systems: physostegia chlorotic mottle virus (submitted to Phytopathology)

Temple, C., Blouin, A.G., Steyer, S., Massart, S. First report of Melon chlorotic spot virus in Belgium and in cultivated sorrel (*Rumex acetosa*) (submitted to Plant disease)

Moubset, O., François, S., Maclot, F., Palanga, E., Julian, C., Claude, L., Fernandez, E.,**Temple, C.,** et al., 2022. Virion-associated nucleic acid-based metagenomics: a decade of advances in molecular characterization of plant viruses. Phytopathology®.

Fontdevila N., Khalili M., Maachi, A., Rivarez, MPS., Rollin J., Salavert, F., **Temple, C.,** et al., 2023. Managing the deluge of newly discovered plant viruses and viroids: an optimized scientific and regulatory framework for their characterization and risk analysis. Front. Microbiol. Sec. Virology

Schoen, R., de Koning, P. P. M., de Krom, C., Oplaat, C., Westenberg, M., Massart, S., **Temple, C.,** et al. 2023. First report of lettuce ring necrosis virus in chili pepper and tomato in Belgium and The Netherlands. Plant Dis.

Oral presentations at national and international conferences

- *72nd International Symposium on Crop Protection (ISCP, Gent, 2021)*

Oral presentation (online):
Biological characterization of an emergent plant virus: Physostegia chlorotic mottle virus

- *18e Rencontres de virologie végétale (RVV, Aussois, 2021)*

Oral presentation:
Characterization of an emergent viral disease on vegetables in Europe Physostegia chlorotic mottle alphanucleorhabdovirus (PhCMoV)

- *International Advances in Plant Virology (IAPV, 2022)*

Oral presentation:
Characterization of an emergent viral disease on vegetables in Europe

- *19e Rencontres de virologie végétale (RVV, Aussois, 2023)*

Oral presentation:
Tomato virus-related risks in diversified vegetable farming systems combining producer's perceptions and virus richness evaluation by High Throughput Sequencing technologies