VIRTIGATION – Emerging viral diseases in tomatoes and cucurbits: Implementation of mitigation strategies for durable disease management

Deliverable 1.4

RESULTS AND ACHIEVEMENTS OF THE VIRTIGATION MULTI-ACTOR APPROACH – PRACTICE ABSTRACTS BATCH 1

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RESULTS AND ACHIEVEMENTS OF THE VIRTIGATION MULTI-ACTOR APPROACH – PRACTICE ABSTRACTS BATCH 1

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LIST OF ABBREVIATIONS

CRISPR - Clustered Regularly Interspaced Shor Palindromic Repeats

D - Deliverable

EIP-AGRI – European Innovation Partnership Agricultural Productivity and Sustainability

IPM – Integrated Pest Management

MA – Multi -actor approach

MOU - Memorandum of Understanding

NKB- National Knowledge Brokers

P. A. – Practice Abstract

PCR – Polymerase Chain Reaction

PepMV-IL – Pepino Mosaic Virus

RT-qPCR - Reverse Transcription quantitative real-time PCR

TMV- Tomato Mosaic Virus

ToBRFV - Tomato Brown Rugose Fruit Virus

ToLCNDV- Tomato Leaf Curl New Delhi Virus

UAS - University of Agricultural Sciences, Bangalore

WP – Work Package

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1 PUBLISHABLE SUMMARY

This deliverable presents a compilation of 14 practice abstracts of the results and knowledge generated during the first period (M1-M18) of VIRTIGATION project.

Meaningful results from first period of the project and summarized in this Practice Abstracts Deliverable are connected to relevant topics on the virosis control, such as

- Transmission of ToLCNDV by different whitefly vectors and reservoir / hosts plants
- IPM control of whitefly on vegetable crops and climate change impact
- Diagnosis, monitoring and symptoms, latent infections
- Tools and methodologies for insects monitoring: eggsplorer
- Disinfection methodologies
- Identification of resistance genes for virosis control

In addition, the results of co-creation activities performed with the help of NKB in different countries during these months are included:

- **Workshops organized with stakeholders** with conclusions from the interactions between stakeholders and VIRTIGATION partners, where main concerns raised such as long times for new varieties development due to gene -editing restrictions, administrative requirements, efficient diagnosis methodologies, good practices in farm management to prevent transmissions

- **Questionnaire to farmers on whiteflies control and distribution** in conventional farms with chemical control and biorational ones with IPM control in Belgium, France, Italy and Spain

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2 EIP-AGRI PRACTICE ABSTRACTS

A "practice abstract" is a short summary of around 1000-1500 characters (word count – no spaces) which describes a main information/recommendation/practice that can serve end-users and stakeholders of the project (farmers, agroindustry company, research community, policy makers, …) with their daily practice. The practice abstracts make innovative knowledge accessible via the EIP-AGRI website for broad dissemination in the common project language (English) and local languages.

VIRTIGATION languages are English, Spanish, French, German, Italian, Dutch and Indian. Although English is the common project language, it's important to have the information available in local languages to provide accessible and understandable information for key stakeholders like farmers.

This deliverable contains ***14 practice abstracts*** that has been produced during the first period by VIRTIGATION partners.

Furthermore, a Zenodo community on "Fighting viral diseases in tomatoes and cucurbits" have been initiated, where complete and extended information from the publications and materials are available: https://zenodo.org/communities/virtigationh2020/?page=1&size=20

The practice abstracts are structured in this deliverable in 2 sections:

1. ***Practice Abstracts from Peer review Publications:***

    - P.A.1. ToLCNDV-Spain is not transmitted by *Trialeurodes vaporariorum* and is inefficiently transmitted by *Bemisia tabaci* MED between zucchini and the wild cucurbit *Ecballium elaterium - available in English / Spanish*
    - P.A.2. Begomoviruses: what is the secret(s) of their success?- *available in English / Spanish*
    - P.A.3. To be seen or not to be seen: Latent infection by tobamoviruses- *available in English / German*
    - P.A.4. Eggsplorer: A rapid plant insect resistance determination tool using an automated whitefly egg quantification algorithm. *Available in English/ Dutch*
    - P.A.5. *Solanum elaeagnifolium* and *S. rostratum* as potential hosts of the tomato brown rugose fruit virus – *Available in English*
    - P.A.6. Combined pest – zoophytophagous predator effects on plants: can Macrolophus pygmaeus mediate the damage caused by Bemisia tabaci on vegetable crops? – *Available in English / Italian*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2. *Practice Abstracts from poster presentations in Conferences/ Congress*

- P.A.7. Early warning system and mitigation strategies for Tomato brown rugose fruit virus, available in *English*
- P.A.8. Optimization of solarization and steaming methods to eradication TMV – available in *English /Spanish*
- P.A.9. Exploring wild Solanum germplasm for resistance to ToBRFV (Tomato Brown Rugose Fruit Virus) - available in *English /Dutch*
- P.A.10. Characterization of tomato leaf curl New Delhi virus infecting cucurbits in France – available in *English /French*
- P.A.11. Effects of *Bemisia tabaci* and *Macrolophus pygmaeus* on morpho-physiological traits of plants – available in *English / Italian*
- P.A.12. The European project "Virtigation": an opportunity to learn more about whitefly pests of vegetable crops and to update on strategies for their control – available in *English / Italian*
- P.A.13. Impact of climate change on a whitefly-transmitted virus – available in *English / German*
- P. A. 14. VIRTIGATION: A network to fight viral diseases – available *in English / German*

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2.1 Practice Abstract 1

TITLE: ToLCNDV-Spain is not transmitted by Trialeurodes vaporariorum and is inefficiently transmitted by Bemisia tabaci MED between zucchini and the wild cucurbit Ecballium elaterium

Begomoviruses constitute a successful group of emerging plant viruses threatening vegetable, root and fiber crops worldwide that are transmitted in nature by whiteflies of the *Bemisia tabaci* complex. Tomato leaf curl New Delhi virus is a paradigmatic example of a begomovirus that has recently emerged in Mediterranean countries after movement from its original location in the Indian subcontinent. The Mediterranean isolates of this virus belong to a novel strain, named "Spain strain", which infects zucchini and other cucurbits but is poorly adapted to tomato. This work aimed to clarify some aspects of the whitefly transmission of tomato leaf curl New Delhi virus.

Practical information for growers:

Our findings indicate that, unlike a recent report on an Indian isolate of the virus, the Mediterranean isolate of tomato leaf curl New Delhi virus is not transmitted by the greenhouse whitefly (Trialeurodes vaporariorum). Furthermore, the most common Bemisia tabaci species in Mediterranean regions is an inefficient vector for transferring this begomovirus between zucchini and the wild cucurbit Ecballium elaterium. These insights suggest that, despite frequent infections, E. elaterium is unlikely to serve as a significant reservoir for this virus strain. Consequently, control measures focusing on the Mediterranean B. tabaci populations and cultivated cucurbits may be more effective for managing disease risk in zucchini fields than targeting wild plant reservoirs.

More information: https://www.mdpi.com/2075-4450/14/4/384

NKB: Mariana B. Lorbach

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: ToLCNDV-Spain no se transmite por *Trialeurodes vaporariorum* y es ineficientemente transmitido por *Bemisia tabaci* MED entre calabacín y la cucurbitácea silvestre Ecballium elaterium

Los begomovirus constituyen un exitoso grupo de virus vegetales emergentes que amenazan los cultivos de hortalizas, tubérculos y fibras en todo el mundo y que se transmiten en la naturaleza por las moscas blancas del complejo Bemisia tabaci. El virus del rizado del tomate de Nueva Delhi es un ejemplo paradigmático de un begomovirus que ha surgido recientemente en los países mediterráneos después del movimiento desde su ubicación original en el subcontinente indio. Los aislamientos mediterráneos de este virus pertenecen a una nueva cepa, denominada "cepa España", que infecta a los calabacines y otras cucurbitáceas pero se adapta mal al tomate. Este trabajo tuvo como objetivo aclarar algunos aspectos de la transmisión por mosca blanca del virus del rizado del tomate de Nueva Delhi.

Información práctica para agricultores:

Nuestros hallazgos indican que, a diferencia de un estudio reciente sobre un aislado indio del virus, el aislado mediterráneo del virus del rizado de la hoja del tomate de Nueva Delhi no es transmitido por la mosca blanca de invernadero (Trialeurodes vaporariorum). Además, la especie más común de Bemisia tabaci en las regiones mediterráneas es un vector ineficaz para transferir este begomovirus entre el calabacín y la cucurbitácea silvestre Ecballium elaterium. Estos resultados sugieren que, a pesar de las infecciones frecuentes, E. elaterium probablemente no actúa como un reservorio significativo para esta cepa del virus. En consecuencia, las medidas de control enfocadas en las poblaciones mediterráneas de B. tabaci y en las cucurbitáceas cultivadas pueden ser más efectivas para gestionar el riesgo de enfermedad en los campos de calabacín que el control de reservorios de plantas silvestres.

Mas información: https://www.mdpi.com/2075-4450/14/4/384

NKB: Mariana B. Lorbach

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.2 Practice Abstract 2

TITLE: Begomoviruses: what is the secret(s) of their success?

Begomoviruses constitute an extremely successful group of emerging plant viruses transmitted by whiteflies of the Bemisia tabaci complex. Hosts include important vegetable, root, and fiber crops grown in the tropics and subtropics. Factors contributing to the ever-increasing diversity and success of begomoviruses include their predisposition to recombine their genomes, interaction with DNA satellites recruited throughout their evolution, presence of wild plants as a virus reservoir and a source of speciation, and extreme polyphagia and continuous movement of the insect vectors to temperate regions. These features as well as some controversial issues (replication in the insect vector, putative seed transmission, transmission by insects other than *B. tabaci*, and expansion of the host range to monocotyledonous plants) will be analyzed in this review.

Practical information for growers:

From a practical perspective, this review provides valuable insights for farmers aiming to manage and prevent begomovirus-related crop losses. By understanding the factors that enhance the spread and adaptability of begomoviruses—including their reliance on whitefly vectors, wild plant reservoirs, and potential alternative transmission pathways—farmers can better implement strategies to monitor and control both vector populations and virus-carrying plants within and surrounding their fields. Proactive surveillance and vector management, especially in regions experiencing milder winter conditions, are key to reducing the risk of viral outbreaks in sensitive crops.

More information:

https://www.cell.com/trends/plant-science/fulltext/S1360-1385(23)00032-8#:~:text=Factors%20contributing%20to%20the%20ever,and%20extreme%20polyphagia%20and%20continuous

NKB: Mariana B. Lorbach

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Los begomovirus: ¿Cuál es el secreto de su éxito?

Los begomovirus constituyen un grupo extremadamente exitoso de virus emergentes de plantas transmitidos por moscas blancas del complejo *Bemisia tabaci*. Los huéspedes incluyen importantes cultivos de hortalizas, tubérculos y fibras que se cultivan en los trópicos y subtrópicos. Los factores que contribuyen a la diversidad y al éxito cada vez mayores de los begomovirus incluyen su predisposición a recombinar sus genomas, la interacción con los satélites de ADN reclutados a lo largo de su evolución, la presencia de plantas silvestres como reservorio de virus y fuente de especiación, así como la polifagia extrema y el movimiento continuo de los insectos vectores a las regiones templadas. En esta revisión se analizan estas características, así como algunos temas controvertidos (replicación en el insecto vector, supuesta transmisión por semillas, transmisión por insectos distintos de *B. tabaci* y expansión del rango de huéspedes a plantas monocotiledóneas).

Información práctica para agricultores:

Desde una perspectiva práctica, esta revisión ofrece información valiosa para los agricultores que buscan gestionar y prevenir pérdidas de cultivos relacionadas con begomovirus. Al comprender los factores que potencian la propagación y adaptabilidad de estos virus, como su dependencia de vectores de mosca blanca, reservorios en plantas silvestres y posibles vías de transmisión alternativas, los agricultores pueden implementar mejor estrategias para monitorear y controlar tanto las poblaciones de vectores como las plantas portadoras de virus dentro y alrededor de sus campos. La vigilancia proactiva y la gestión de vectores, especialmente en regiones con inviernos más suaves, son clave para reducir el riesgo de brotes virales en cultivos sensibles.

Mas información:

https://www.cell.com/trends/plant-science/fulltext/S1360-1385(23)00032-8#:~:text=Factors%20contributing%20to%20the%20ever,and%20extreme%20polyphagia%20and%20continuous

NKB: Mariana B. Lorbach

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.3 Practice Abstract 3

TITLE: To be seen or not to be seen: Latent infection by tobamoviruses

This review looks at the group of tobamoviruses to which tomato brown rugose fruit virus belongs. Some tobamoviruses cause latent infection in their hosts, which means that no obvious symptoms are visible. This has implicitations for diagnostics as multiple infections with latent and non-latent viruses may only focus on the symptom-inducing viruses. Furthermore, latent viruses may affect symptomology or replication of co-infecting viruses which may lead to more severe symptoms on host plants.

Practical information for growers:

Farmers should be aware that they crop can be infected with latent viruses even though no symptoms are visible. They shoud use clean and healthy seeds and young plants and check their crops regularly for (latent) virus infections.

More information:

https://www.mdpi.com/2223-7747/11/16/2166

NKB: Guadalupe López

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Sichtbar oder unsichtbar - Latente Infektionen durch Tobamoviren

Dieser Review betrachtet die Tobamovirusgruppe, eine Gruppe von Viren, zu denen auch das tomato brown rugose fruit virus zählt. Einige Tobamoviren rufen latente Infektionen hervor, d.h., auf den Wirtspflanzen sind keine oder nur sehr milde Symptome sichtbar. Dieses stellt Diagnostiker vor besondere Herausforderungen, da in Mischinfektionen von latenten und nicht-latenten Viren oft der Fokus auf den symptom-verursachenden Viren liegt. Darüberhinaus können latente Viren auch Einfluß auf die Symptome oder Replizierung von co-infizierenden Viren haben, was zu stärkeren Symptomen auf Wirtspflanzen führen kann.

Praktische Informationen für Landwirte:

Produzenten sollten sich bewußt sein, dass ihre Pflanzen latent infiziert sein könnten. Sie sollten daher sauberes und gesundes Saatgut bzw. Pflanzgut verwenden und ihre Pflanzen regelmäßig auf (latente) Virusinfektionen testen.

Mehr Informationen:

https://www.mdpi.com/2223-7747/11/16/2166

NKB: Guadalupe López

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.4 Practice Abstract 4

TITLE: Eggsplorer: a rapid plant–insect resistance determination tool using an automated whitefly egg quantification algorithm

Background Counting of insects on plants is crucial in plant breeding for insect resistance, to determine infestation in the field, or for assessment of crop protection products. The traditional method of visually counting insect (larvae, eggs) using a microscope is time-consuming and requires expertise. For instance, whiteflies, a common pest on various crops, can lay up to hundreds of eggs on a single leaf within a few days. An automated and rapid method for quantifying insect eggs can save time and human resources. **Results** Researchers created a tool called Eggsplorer, which automates the counting of whitefly eggs. Images of leaves with whitefly eggs were collected using a custom-built imaging system. These images were used to train a deep learning model that can detect and count the eggs accurately. The model was then integrated in a user-friendly web-based application. In testing, Eggsplorer achieved a counting accuracy of nearly 94% when compared to manual counts. It proved to be a reliable method for determining the resistance and susceptibility of different plants to whitefly infestation.

Practical recommendation for growers:

Eggsplorer presents a new method for fast automatic determination of insects eggs on plants, that is easily accessible for users in a web-based application. Eggsplorer could be integrated into mobile phone platforms allowing users to collect data on the go and receive real-time advice on pest infestation. Furthermore, this innovation can be further developed to analyze not only eggs but other developmental stages of other insects on different plants.

More information:

https://plantmethods.biomedcentral.com/articles/10.1186/s13007-023-01027-9

NKB: Rene Van der Vlugt

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Eggsplorer: een snelle methode om automatisch plaagontwikkeling te bepalen

Het tellen van insecten op planten is essentieel bij plantenveredeling en het beoordelen van gewasbescherming. De traditionele methode van visueel tellen van insecteneieren met behulp van een microscoop is tijdrovend en vereist expertise. Om dit probleem aan te pakken, is er een nieuwe geautomatiseerde tool ontwikkeld genaamd Eggsplorer. Om deze tool te ontwikkelen zijn foto's van bladeren met wittevliegeieren verzameld met behulp van een op maat gemaakt camerasysteem. Deze afbeeldingen zijn gebruikt om een deep learning model te trainen voor nauwkeurige detectie en tellen van de eieren. Het model is vervolgens geïntegreerd in een gebruiksvriendelijke web-app, waar snel en nauwkeurig fotos geupload en geanalyseerd kunnen worden. Bij testen behaalde Eggsplorer een nauwkeurigheid van bijna 94% bij het tellen in vergelijking met experts. Met de methode konden betrouwbaar resistentie en vatbaarheid van verschillende planten voor wittevliegbesmetting bepaald worden.

Praktisch advise voor boeren:

Eggsplorer kan gebruikt worden voor snelle en automatische bepaling van insecteneieren op planten, gemakkelijk toegankelijk via een web-based app. Integratie in een mobiele app zou gegevensverzameling onderweg mogelijk maken en gebruikers real-time van advies kunnen voorzien over plaag-ontwikkeling. Bovendien kan deze innovatie verder worden ontwikkeld om ook andere ontwikkelingsstadia van insecten op verschillende planten te analyseren.

Meer informatie:

https://plantmethods.biomedcentral.com/articles/10.1186/s13007-023-01027-9

NKB: Rene Van der Vlugt

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.5 Practice Abstract 5

TITLE: Solanum elaeagnifolium and S. rostratum as potential hosts of the tomato brown rugose fruit virus

Invasive weeds cause significant crop yield and economic losses in agriculture. The highest indirect impact may be attributed to the role of invasive weeds as virus reservoirs within commercial growing areas. The new tobamovirus tomato brown rugose fruit virus (ToBRFV), first identified in the Middle East, overcame the Tm-22 resistance allele of cultivated tomato varieties and caused severe damage to crops. In this study, we determined the role of invasive weed species as potential hosts of ToBRFV and a mild strain of pepino mosaic virus (PepMV-IL). Of newly tested weed species, only the invasive species *Solanum elaeagnifolium* and *S. rostratum* were susceptible to ToBRFV infection. S. rostratum was also susceptible to PepMV-IL infection. No phenotype was observed on ToBRFV-infected *S. elaeagnifolium* grown in the wild or following ToBRFV inoculation. S. rostratum plants inoculated with ToBRFV contained a high ToBRFV titer compared to ToBRFV-infected S. elaeagnifolium plants. Mixed infection with ToBRFV and PepMV-IL of S. rostratum plants, as well as S. nigrum plants (a known host of ToBRFV and PepMV), displayed synergism between the two viruses, manifested by increasing PepMV-IL levels. Additionally, when inoculated with either ToBRFV or PepMV-IL, disease symptoms were apparent in S. rostratum plants and the symptoms were exacerbated upon mixed infections with both viruses. In a bioassay, ToBRFV-inoculated *S. elaeagnifolium, S. rostratum* and *S. nigrum* plants infected tomato plants harboring the Tm-22 resistant allele with ToBRFV. The distribution and abundance of these Solanaceae species increase the risks of virus transmission between species.

Practical information for growers:

It is most important to eradicate all Solanaceae weed species grown adjacent to the tomato plants inside the greenhouse and in the surrounding area.

More information:

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0282441

NKB: Guadalupe López

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.6 Practice Abstract 6

TITLE: Can Macrolophus pygmaeus mediate the damage caused by Bemisia tabaci on vegetable crops?

Today, pest management based on biological control is the most sustainable alternative to pesticide use. The whitefly Bemisia tabaci is one of the key pests negatively impacting yield and quality of vegetable crops; while the predator Macrolophus pygmaeus is one of the main natural enemies widely used for its control, although it can sometimes behave as a pest, causing damage to plants. In this study, the impact of M. pygmaeus as a plant feeder has been investigated, by analyzing the combined impact of the whitefly and the predator bug on potted eggplants under laboratory conditions. Results show no statistical differences between the heights of plants infested by the whitefly or by both insects compared with noninfested control plants. However, indirect chlorophyll content, photosynthetic performance, leaf area, and shoot dry weight were all greatly reduced in plants infested only by B. tabaci, compared with those infested by both the pest and its predator or with noninfested control plants. Contrarily, root area and dry weight values were more reduced in plants exposed to both insects, compared to those infested only by the whitefly or to noninfested plants (the latter showing the highest values). These results show how M. pygmaeus can significantly reduce the damage caused by B. tabaci to host plants, although its effects on the belowground part of the plants remains unclear. Anyway, from a practical point of view and given the overall positive effect of the predator, both for its control of the whitefly and reduction of negative effects on crops, its release during the early stages of B. tabaci infestation in greenhouse environments is recommended.

Practical information for growers:

It is essential for farmers to thoroughly understand the behavior of natural enemies, particularly zoo-phytophagous predators, which interact not only with prey but also with plants. These predators often spontaneously colonize greenhouses with horticultural crops in southern Sicily, and if present in excessive quantities, they can cause damage to the plants. So, their correct identification and a deep understanding of these dynamics enables a more balanced and sustainable management of B. tabaci infestations.More information:

https://www.mdpi.com/2075-4450/14/2/164

NKB: Guadalupe López

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Può Macrolophus pygmaeus mediare i danni causati da Bemisia tabaci sulle colture orticole?

Al giorno d'oggi, la gestione dei parassiti basata sul controllo biologico è l'alternativa più sostenibile all'uso di agrofarmaci. L'aleirode Bemisia tabaci costituisce uno dei parassiti chiave che incidono negativamente sulla resa e sulla qualità delle colture ortive; invece, il miride Macrolophus pygmaeus è considerato uno dei principali nemici naturali ampiamente utilizzati per il suo controllo. Talvolta tale predatore può causare danni alle colture, pertanto in questo studio è stato esaminato l'impatto combinato di B. tabaci e del predatore su piante di melanzana in condizioni di laboratorio. I risultati non hanno mostrato differenze statistiche tra le altezze delle piante infestate dal parassita o da entrambi gli insetti rispetto alle piante non infestate. Tuttavia, il contenuto indiretto di clorofilla, la prestazione fotosintetica, l'area fogliare e il peso secco dei germogli sono stati maggiormente modificati nelle piante infestate solo da B. tabaci, rispetto a quelle infestate sia dall'alaeirode che dal suo predatore o rispetto alle piante non infestate. Contrariamente, i valori dell'area e del peso secco delle radici sono risultati inferiori nelle piante esposte a entrambe le specie di insetti, rispetto a quelle infestate solo dall'aleirodide o alle piante non infestate. Questi risultati mostrano come il predatore possa ridurre significativamente i danni causati alle piante ospiti dalla presenza di B. tabaci, anche se il suo effetto sulla parte ipogea delle piante rimane ancora poco chiaro. Tuttavia, dato l'effetto complessivamente positivo del miride predatore sulle colture, da un punto di vista pratico, viene raccomandato il suo rilascio durante le prime fasi di infestazione di B. tabaci negli ambienti serricoli.

Informazioni pratiche per gli agricoltori

È fondamentale che gli agricoltori comprendano a fondo il comportamento dei nemici naturali, in particolare dei predatori zoofitofagi, che non si limitano a interagire con le prede, ma anche con le piante. Questi predatori, infatti, spesso colonizzano spontaneamente le serre orticole del sud della Sicilia e, se presenti in quantità eccessive, possono arrecare danni alle colture. Pertanto una loro corretta identificazione e una conoscenza approfondita di tali dinamiche consente quindi una gestione più equilibrata e sostenibile delle infestazioni di B. tabaci.

Mas Informazione:

https://www.mdpi.com/2075-4450/14/2/164

NKB: Guadalupe López

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.7 Practice Abstract 7

TITLE: Early warning system and mitigation strategies for Tomato brown rugose fruit virus

Tomato brown rugose fruit virus (ToBRFV) poses a huge threat to commercial tomato cultivation worldwide. Due to its high persistence and easy mechanical transmission, ToBRFV is extremely hard to get rid of once it enters the greenhouse. Current management strategies therefore centre around preventive measures, such as improved hygiene, in an attempt to keep the virus out of the crop. Early detection of the virus can also play an important role. Our research shows that ToBRFV circulates in greenhouse water systems and can be detected in drain water samples before the plants start showing symptoms. We illustrate that drain water can be used to monitor ToBRFV outbreaks.

Practical information for growers:

This technique can serve as an early warning system, allowing growers to take timely action against the virus and limit the spread of the infection to a minimum.

More information:

NKB: Marlies Huysmans

https://zenodo.org/record/7928630

Presented in:

- International Symposium of Plant Virus Epidemiology, Madrid (Spain), June 2022
- Tomato Brown Rugose Fruit Virus Research Symposium, Toronto (Canada), August 2022

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.8 Practice Abstract 8

TITLE: Optimization of solarization and steaming methods to eradication TMV

TECNOVA (TEC, Spain) and Landwirtschaftskammer Nordrhein-Westfalen (LNW, Germany) work together in the task "Optimization of eradication methods after tobamovirus outbreaks" of VIRTIGATION project. Both institutes are working on the validation of solarization and steaming methods to eradicate tobamoviruses in contaminated cocopeat bags by TMV (Tobacco mosaic virus). For solarization, TEC has used a transparent 37.5 µm think polyethylene plastic during 60 days (from September to November to try simulate the summer in other countries in Europe) with reordered temperatures between 15.2 to 46.2°C. LNW is testing two steaming protocols: (1) 90 °C during 20 min and (2) at 90 °C during 40 min. In order to test the success of both methods, the disinfected bags were used to growth new plants. In addition, it has tried to discern if the substrate must necessarily be free of crop residues or not. The results of the solarization have been very promising. All tomato plants grew up symptomless in solarized bags. Only in one of the six lines (temperature > 40°C during 10-13 days) TMV were detected by PCR. Moreover, in bags with infected material (roots and leaves), the maximum temperature was until 4°C higher than in bags with just substrate. Incorporate vegetal material helped to increase the number of days with high temperature (> 40°C), from 7-13 to 14-34 days in substrate bags without and with infected material, respectively.

Practical information for growers:

The steaming results are coming soon in the following moths. After steaming it has been detected: (i) plants in the non-steamed bags showed better fitness than the steamed variants; (ii) growth of mold fungi could be observed on the substrate and (iii) substrate bags must be used immediately and cannot be stored for a while.

More information:

NKB: Mariana B. Lorbach

Presented in :

- Symposium soils disinfection, Almería, June 2022

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Optimización de los métodos de solarización y vaporización para la erradicación de TMV

TECNOVA (TEC, España) y Landwirtschaftskammer Nordrhein-Westfalen (LNW, Alemania) colaboran en la "Optimización de métodos de erradicación de tobamovirus" del proyecto VIRTIGATION. Los institutos están trabajando en la validación métodos de solarización y esterilización por vapor para erradicar tobamovirus en sacos de fibra de coco contaminadas por TMV (Tobacco mosaic virus). Para la solarización se ha utilizado un plástico de polietileno transparente de 37,5 µm durante 60 días (de septiembre a noviembre intentando simular el verano en países europeos) con temperaturas entre 15,2 y 46,2°C. LNW está probando dos protocolos de vaporización: (1) 90°C, 20 min y (2) 90°C, 40 min. Para probar el éxito de ambos métodos, los sacos desinfectados se usaron para cultivar nuevas plantas. Además, se pretende discernir si el sustrato debe estar libre de residuos de cosecha o no. Los resultados de la solarización han sido muy prometedores. Todas las plantas de tomate crecieron sin síntomas en los sacos solarizados. Únicamente en una de las seis líneas (temperatura > 40°C durante 10-13 días) se detectó TMV mediante PCR. Además, en los sacos con material infectado (raíces y hojas), la temperatura máxima fue hasta 4°C mayor que en las bolsas con solo sustrato. La incorporación de material vegetal ayudó a aumentar el número de días con temperatura alta (> 40°C), de 7-13 a 14-34 días en los sacos sin y con material infectado, respectivamente.

Informacion práctica para agricultores:

Se han detectado los siguientes resultados prácticos: (i) las plantas en los sacos no vaporizados mostraron mayor vigorosidad; (ii) tras el tratamiento se ha observado el crecimiento de moho en el sustrato y, por lo tanto, (iii) los sacos deben usarse de inmediato y no almacenarse por un tiempo. Este ensayo se encuentra todavía en ejecución.

Más información:

NKB: Mariana B. Lorbach

Poster presentado en

- Symposium soils disinfection, Almería, June 2022

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.9 Practice Abstract 9

TITLE: Exploring wild Solanum germplasm for resistance to ToBRFV (Tomato Brown Rugose Fruit Virus)

ToBRFV is a newly emerged virus that causes severe losses in tomato production. Within five years, ToBRFV infections were reported in Asia, North America and Europe. ToBRFV belongs to tobamovirus genus, however all the available tomato resistant varieties used for tobamoviruses, such as TMV and ToMV, cannot halt infections caused by this new virus. The severity of this disease, its rapid spread, and the scarcity of resistant cultivars make this virus a global threat for tomato production. Our objective is to find ToBRFV resistance in wild Solanum accessions and introgress this trait into tomato cultivars reaching the first step for creating modern resistant tomato varieties. So far, we have screened 75 Solanum accessions from which two S. pennellii found resistant to ToBRFV. We have mapped the resistance locus and developed molecular markers. The resistance sources can used in breeding to introgress this locus into cultivated tomato, where the marker can be applied for marker assisted selection.

Practical information for growers:

For growers, the developed markers can be used to check whether the resistance locus is present in their cultivated tomato varieties. Or, growers can ask seed companies for information about the presence of this resistance locus in the cultivars that they have bought.

More information:

NKB: Rene Van der Vlugt

Presented in :

- XVII SOLANACEAE2022

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Zoeken naar resistentie tegen Tomato brown rugose fruit virus (ToBRFV)

ToBRFV is een nieuw virus dat ernstige schade en verliezen veroorzaakt in de tomatenteelt. Binnen vijf jaar na de eerste vondstren van het virus in Jordanië en Israël werden ToBRFV-infecties gemeld in Azië, Noord-Amerika en Europa. ToBRFV behoort tot de groep van de tobamovirussen, maar geen van de tomatenrassen die resistent zijn tegen tobamovirussen zoals TMV en ToMV, kan de infecties die door dit nieuwe virus worden veroorzaakt stoppen. De ernst van deze ziekte, de snelle verspreiding ervan en het gebrek aan resistente cultivars maken dit virus tot een wereldwijde bedreiging voor de tomatenteelt. Ons doel is ToBRFV-resistentie te vinden in wilde Solanum-soorten en deze eigenschap in commerciele tomatencultivars te introduceren, waarmee we de eerste stap zetten naar het creëren van moderne resistente tomatenrassen. Tot nu toe hebben we 75 Solanum-accessies gescreend, waarvan twee S. pennellii-accessies resistent werden bevonden tegen ToBRFV. Wij hebben de resistentielocus in kaart gebracht en moleculaire markers ontwikkeld. De resistentiebronnen kunnen bij de veredeling worden gebruikt om deze locus in gecultiveerde tomaten te introduceren, waar de marker kan worden toegepast voor door markers ondersteunde selectie.

Praktisch advise voor boeren:

Voor telers kunnen de ontwikkelde markers worden gebruikt om te controleren of het resistentielocus aanwezig is in hun geteelde tomatenrassen. Of telers kunnen zaadbedrijven om informatie vragen over de aanwezigheid van dit resistentielocus in de cultivars die zij hebben gekocht.

Meer informatie:

NKB: Rene Van der Vlugt

Poster presentado en

- XVII SOLANACEAE2022

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.10 Practice Abstract 10

TITLE: Characterization of tomato leaf curl New Delhi virus infecting cucurbits in France

Tomato leaf curl New Delhi virus (ToLCNDV) is now emerging in the Mediterranean Basin, starting from Spain in 2012. It has been observed for the first time in France in 2020 in Gard and Bouches-du-Rhône. Mediterranean ToLCNDV is a bipartite begomovirus, causing leaf curl disease mainly in zucchini and others cucurbits, and genetically distinct from Asian (Indian)-ToLCNDV that has a broader host range. The cryptic species of whitefly Bemisia tabaci is the main insect vector of ToLCNDV while mechanical inoculation is also possible. Previous studies indicate that the Mediterranean clade represents a homogenous population, probably originating from a single introduction. We established a suitable protocol of inoculation and performed biological and molecular characterization of the French isolates in order to estimate their risks of emergence and their potential agronomic impact. Symptom observation of French ToLCNDV isolates on melon and zucchini showed two different types so-called “severe” and “recovery”. French ToLCNDV was found to be transmissible by Bemisia tabaci as expected but not by the greenhouse whitefly Trialeurodes vaporariorum.

Practical information for growers:

Host range analysis in experimental conditions suggest that Bryony and Tomato are susceptible to French and Spanish ToLCNDV and may constitute virus reservoirs, what should be taken into consideration for virus control. Our results expand the knowledge on this emerging virus and offer perspectives for shaping the future plant disease management.

More information:

NKB: Cecile Desbiez

Presented in :

- ISPVE 2022
- Rencontres de Virologie Végétale RVV2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Caractérisation du tomato leaf curl New Delhi virus infectant les cucurbitacées en France

Le ToLCNDV est un virus émergent dans le Bassin Méditerranéen, où il a d'abord été observé en Espagne en 2012. En France, il a été observé pour la première fois en 2020 dans le Gard et les Bouches-du-Rhône. Le ToLCNDV Méditerranéen est un begomovirus bipartite, causant des déformations foliaires surtout chez les cucurbitacées, et génétiquement distinct du ToLCNDV Asiatique (Indien) qui infecte une gamme de plantes plus large. Le complexe d'espèces d'aleurodes Bemisia tabaci est le principal vecteur du ToLCNDV; le virus peut être transmis mécaniquement de façon peu efficace. Des études précédentes ont montré que le ToLCNDV constitue une population génétiquement homogène, probablement issue d'une introduction unique. Nous avons développé des travaux pour caractériser les isolats français au niveau biologique et moléculaire, en vue d'estimer leurs risques d'émergence et leur impact agronomique potentiel. L'observation des symptômes sur melon et courgette a révélé deux types différents, « sévère » ou « avec récupération » selon les isolats. Des expériences de transmission par insecte ont montré que le ToLCNDV Français est transmis par B. tabaci comme attendu mais pas par l'aleurode des serres Trialeurodes vaporariorum.

Informations pratiques pour les agriculteurs

Des résultats en conditions expérimentales indiquent que la tomate et la bryone sont des hôtes potentiels du ToLCNDV et peuvent constituer des réservoirs viraux. Nous résultats améliorent la connaissance de ce virus émergent en vue de mieux gérer la maladie à l'avenir.

Plus d´informations:

NKB: Cecile Desbiez

Affiche prénsentée dans:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.11 **Practice Abstract 11**

TITLE: Effects of *Bemisia tabaci* and *Macrolophus pygmaeus* on morpho-physiological traits of plants

Whiteflies (Hemiptera: Aleyrodidae) are critical pests attacking many cultivated plants in almost all areas of the world. Among them, Bemisia tabaci (Gennadius) is a global pest that causes significant losses to a wide variety of crops by affecting plants development. To control B. tabaci infestations, the release of natural enemies has become increasingly important as an ecologically safe and effective biological control method and, among predators used, the mirid bug Macrolophus pygmaeus (Rambur) plays a primary role. Anyway, due to its habits to feed secondarily also on plants, a wrong application rate of this predator can also make this beneficial a threat; and this is why a better understanding is needed of the role that M. pygmaeus plays on crops, evaluated in a holistic way. To this aim, and in order to deepen knowledge on the impact that whiteflies alone or combined with M. pygmaeus may have on vegetable solanaceous crops (with special emphasis to tomato and eggplant), the main morphological (total height, dry weights, leaf area) and physiological (photosynthetic performance, indirect chlorophyll content) parameters of the plants were analysed in three different conditions: i) healthy plants; ii) plants infested by the pest; iii) plants with both pest and predator. At the experimental conditions and the insect densities adopted, results show a variable susceptibility by different plant species to B. tabaci and a significant reduction induced by M. pygmaeus in negative effects caused by the whitefly, confirming his validity as biological control agent against B. tabaci.

Practical information for growers:

In conclusion, this predator is able to suppress B. tabaci populations, significantly reducing the need for chemical molecules and consequently allowing farmers to preserve ecosystems, prevent pest resistance to insecticides, and protect human and environmental health, ensuring more sustainable agricultural production.

More information:

NKB: Guadalupe López

Presented in :

- XXVII Italian National Congress of Entomology, 12-16 June 2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Effetti di *Bemisia tabaci* e *Macrolophus pygmaeus* sui tratti morfo-fisiologici delle piante

Gli Aleirodidi sono insetti fitofagi che attaccano molte piante coltivate in quasi tutto il mondo. Tra questi, Bemisia tabaci (Gennadius) è un parassita globale che causa perdite significative a un'ampia varietà di colture, influenzando lo sviluppo delle piante. Per controllare le sue infestazioni, il rilascio di nemici naturali è diventato sempre più importante come metodo di controllo biologico ecologicamente sicuro ed efficace e, tra i predatori utilizzati, il miride Macrolophus pygmaeus (Rambur) ha un ruolo primario. Tuttavia, per i suoi costumi zoofitofagi, un'applicazione non corretta di questo predatore può renderlo una minaccia per le piante; ed è per questo che è necessaria una migliore comprensione del ruolo svolto da M. pygmaeus, valutato in modo olistico. Per approfondire le conoscenze sull'impatto che B. tabaci da sola o in combinazione con M. pygmaeus può avere sulle solanacee orticole (soprattutto pomodoro e melanzana), i principali parametri morfologici (altezza totale, peso secco, superficie fogliare) e fisiologici (rendimento fotosintetico, contenuto indiretto di clorofilla) delle piante sono stati analizzati in tre diverse condizioni: i) piante sane; ii) piante infestate dall'aleirode; iii) piante con aleirode e predatore insieme. Alle condizioni sperimentali e alle densità di insetti adottate, i risultati mostrano una suscettibilità variabile delle diverse piante a B. tabaci e una significativa riduzione indotta da M. pygmaeus degli effetti negativi causati dall'aleirode, confermandone la validità quale agente di controllo biologico contro B. tabaci.

Informazioni pratiche per gli agricoltori

In conclusione, l'impiego di questo predatore contribuisce significativamente a contenere le popolazioni di B. tabaci, riducendo la necessità di utilizzare le molecole chimiche e consentendo di conseguenza agli agricoltori di preservare gli ecosistemi, prevenire la resistenza dei parassiti agli insetticidi e proteggere la salute umana e ambientale, garantendo una produzione agricola più sostenibile.Mas Informazione:

NKB: Guadalupe López

Presentato en :

- XXVII Italian National Congress of Entomology, 12-16 June 2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.12 Practice Abstract 12

TITLE: The European project "Virtigation": an opportunity to learn more about whitefly pests of vegetable crops and to update on strategies for their

The project VIRTIGATION, funded by the European Union under the Horizon 2020 programme, involves 25 partners from 12 different countries, and aims to develop solutions for the control of emerging viruses on cucurbits and tomatoes caused by begomoviruses and tobamoviruses (the first ones transmitted by insects). Main entomological objectives of the project are to: i) understand plant-virus-vector interactions; ii) identify ecological factors that favour outbreaks of infections; iii) investigate biology of vector insects and their virus transmission efficiency under climate change conditions; iv) enhance and optimize natural resistance, especially for lower attractiveness of plants to vector insects; v) develop solutions for the integrated control of the viruses and their vectors. Within this project, the University of Catania (Department of Agriculture, Food and Environment – Di3A) will have to: a) contribute to a survey in various partner countries on methods used for whitefly control, especially *Bemisia tabaci*; b) coordinate field trials to evaluate the efficacy of new plant extracts with insecticidal action, also analysing their secondary effects on natural enemies and pollinators; c) carry out field trials with the most promising accessions for their resistance to *B. tabaci* MED; d) evaluate the combination of different approaches for whitefly control.

Practical information for growers:

By advancing the study of insect vectors of viruses, the results will offer valuable techniques and control measures to prevent future epidemics, particularly in the context of climate change. VIRTIGATION's multi-actor approach, which prioritizes the needs of farmers, will thus contribute to defining research activities and developing mitigation strategies for these challenges related to agriculture.

More information:

NKB: Guadalupe López

Presented in :

- XXVII Italian National Congress of Entomology, 12-16 June 2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Il progetto europeo "Virtigation": un'occasione per conoscere meglio gli aleirodi parassiti delle colture orticole e per aggiornarsi sulle strategie del loro controllo

Il progetto VIRTIGATION, finanziato dall'Unione Europea nell'ambito del programma Horizon 2020, coinvolge 25 partner provenienti da 12 paesi diversi, e mira a sviluppare soluzioni per il controllo dei virus emergenti su cucurbitacee e pomodoro causati da begomovirus e tobamovirus (i primi trasmessi da insetti). I principali obiettivi entomologici del progetto sono: i) comprendere le interazioni pianta-virus-vettore; ii) individuare i fattori ecologici che favoriscono l'insorgere di infezioni virali; iii) studiare la biologia degli insetti vettori e la loro efficienza di trasmissione dei virus in condizioni di cambiamento climatico; iv) potenziare e ottimizzare la resistenza naturale, soprattutto per la minore attrattività delle piante nei confronti degli insetti vettori; v) sviluppare soluzioni per il controllo integrato dei virus e dei loro vettori. Nell'ambito di questo progetto, l'Università degli Studi di Catania (Dipartimento di Agricoltura, Alimentazione e Ambiente – Di3A) dovrà: a) contribuire a un'indagine in vari paesi partner sui metodi utilizzati per il controllo degli aleirodidi, con particolare riferimento a Bemisia tabaci; b) coordinare prove di campo per valutare l'efficacia di nuovi estratti vegetali ad azione insetticida, analizzandone anche gli effetti secondari sui nemici naturali e sugli impollinatori; c) effettuare prove di campo con le accessioni più promettenti per la loro resistenza a B. tabaci MED; d) valutare la combinazione di diversi approcci per il controllo degli aleirodidi vettori di virus.

Informazioni pratiche per gli agricoltori

Approfondendo lo studio degli insetti vettori di virus, i risultati ottenuti offriranno tecniche e misure di controllo utili per prevenire future epidemie, soprattutto nel contesto dei cambiamenti climatici. L'approccio multi-attore di VIRTIGATION, che dà priorità alle esigenze degli agricoltori, contribuirà a definire le attività di ricerca e a sviluppare strategie di mitigazione per queste sfide legate all'agricoltura.

Mas Informazione:

NKB: Guadalupe López

Presentato en :

- XXVII Italian National Congress of Entomology, 12-16 June 2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.13 Practice Abstract 13

TITLE: Impact of climate change on a whitefly-transmitted virus

Bemisia tabaci (Hemiptera: Aleyrodidae) is a significant pest worldwide, causing damage to plants both directly and indirectly by transmitting numerous economically important viruses. The Tomato Leaf Curl New Delhi Virus (ToLCNDV), transmitted by whiteflies from the *Bemisia tabaci* complex, spread from Southeast Asia to the Mediterranean region in the early 2000s. ToLCNDV affects cucurbits and has caused significant economic losses in Europe, particularly in Greece, Italy, Spain, Portugal, and recently in France. Climate change is expected to exacerbate the spread of *B. tabaci* and its associated diseases. To better understand how climate change will affect the epidemiology of ToLCNDV in cucurbits, LIST researchers are testing how changes in temperature, humidity, and CO2 concentration in climatic chambers will affect the acquisition and inoculation of ToLCNDV *in B. tabaci,* as well as the gene expression of target genes in the whitefly vector. The experiments aim to shed light on an unexplored topic and help address future challenges in plant protection.

Practical information for growers:

The results collected from the experiment will help growers face the threats posed by the warmer future climate, which is expected to increase the whitefly efficiency in acquiring and transmitting the virus, exacerbating its spread. This shift may worsen disease patterns, making regular pest monitoring and proactive management essential to protect crops effectively.

More information:

NKB: Guadalupe López

Presented in :

- DGaaE Entomology Congress 2023 (Bolzano, Italy)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: Auswirkungen des Klimawandels auf ein von der Weißen Fliege übertragenes Virus

Bemisia tabaci (Hemiptera: Aleyrodidae) ist weltweit ein bedeutender Schädling, der sowohl direkt durch Saugschaden als auch indirekt durch die Übertragung zahlreicher wirtschaftlich wichtiger Viren Schäden an Pflanzen verursacht. Das Tomato Leaf Curl New Delhi Virus (ToLCNDV), das von Weißen Fliegen aus dem Bemisia tabaci-Komplex übertragen wird, breitete sich in den frühen 2000er Jahren von Südostasien bis in den Mittelmeerraum aus. ToLCNDV befällt Kürbisgewächse (z.B. Zuchini) und hat in Europa erhebliche wirtschaftliche Verluste verursacht, insbesondere in Griechenland, Italien, Spanien, Portugal und seit kurzem auch in Frankreich. Es wird erwartet, dass der Klimawandel die Ausbreitung von B. tabaci und der von ihr übertragenen Krankheitenen noch verschlimmern wird. Um besser zu verstehen, wie sich der Klimawandel auf die Epidemiologie von ToLCNDV bei Kürbisgewächsen auswirken wird, testen die LIST-Forscher, wie sich Veränderungen der Temperatur, der Luftfeuchtigkeit und der CO2-Konzentration in Klimakammern auf die Aufnahme von ToLCNDV durch das Schadinsekt und dessen Weitergabe, sowie auf die Genexpression von Zielgenen in der Weißen Fliege auswirken. Die Experimente sollen die Wechselwirkungen zwischen Schadinsekt und Virus charakterisieren und dazu beitragen, zukünftige Herausforderungen im Pflanzenschutz anzugehen.

Praktische Informationen für Landwirte

Die Ergebnisse aus dem Experiment werden den Landwirten helfen, sich den Bedrohungen durch das zukünftig wärmere Klima zu stellen, das voraussichtlich die Effizienz der Weißen Fliege bei der Aufnahme und Übertragung des Virus erhöhen und dessen Verbreitung in Beständen von Kulturpflanzen verschärfen wird. Diese Veränderung könnte Epidemien von Pflanzenkrankheiten verschlimmern, sodass regelmäßiges Schädlingsmonitoring und proaktive Maßnahmen entscheidend für den wirksamen Pflanzenschutz sein werden.

Mehr Informationen:

NKB: Guadalupe López

Präserntierte es :

- XXVII Italian National Congress of Entomology, 12-16 June 2023

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

2.14 Practice Abstract 14

TITLE: VIRTIGATION: A Network to fight Viral Diseases

VIRTIGATION is a multi-actor project on a mission to protect tomatoes and cucurbits in Northern Europe and the Mediterranean Basin. Together with partners from key EU
neighbouring areas – Morocco, Israel and India – the VIRTIGATION project aims to develop a set of bio-based solutions to safeguard tomatoes and cucurbits from emerging viral diseases.
Specifically, VIRTIGATION addresses emerging viral diseases caused by the begomovirus ToLCNDV (Tomato leaf curl New Delhi Virus, transmitted by whiteflies) and the tobamovirus ToBRFV (Tomato brown rugose fruit virus, mechanically transmitted).

Practical information for growers:

In particular practice-relevant measures (recommendations for hygiene and desinfection, using mild strain cross-protecting) will be developed to protect these plants.

More information:

NKB: Guadalupe López

Presented in :

- 62. Deutschen Pflanzenschutztagung

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

TITLE: VIRTIGATION: Ein Netzwerk gegen Viruserkrankungen

VIRTIGATION ist ein Multi-Akteur-Projekt mit dem Ziel Tomaten und Kürbisgewächse vor aufkommenden Viruskrankheiten in Europa und dem Mittelmeerraum zu schützen. Gemeinsam mit Partnern aus europäischen Ländern sowie Marokko, Israel und Indien wird versucht eine Reihe von biobasierten Lösungen zu entwickeln.

Konkret geht es bei VIRTIGATION um neu auftretende Viruskrankheiten, die durch das

Begomovirus ToLCNDV (Tomato leaf curl Neu- Delhi-Virus, Übertragung durch Weiße Fliegen) und das Tobamovirus ToBRFV (Tomato brown rugose fruit virus, mechanische Übertragung) verursacht werden.

Praktische Informationen für Landwirte:

Ziel ist es, insbesondere praxisnahme Maßnahmen (Empfehlungen zur Hygiene und Desinfektion, die Nutzung von Prämunisierung) zu entwickeln, um den Anbau von Gurken und Tomaten zu gewährleisten.

Mehr Informationen:

NKB: Guadalupe López

Präserntierte es :

- 62. Deutschen Pflanzenschutztagung

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

3 SEMINARS AND WORKSHOPS INTERACTIONS

VIRTIGATION partners have been actively involved in several actions to promote interaction with stakeholders, present the project research program and inform about the latest results.

The following actions have been performed:

3.1. VIRTIGATION Workshop with stakeholders in Almería (Spain), organized by AA in March 2022 in TECNOVA headquarters.

VIRTIGATION gathered more than 30 experts in agriculture, plant pathology and plant breeding from across the value chain. With this workshop, VIRTIGATION research partners and stakeholders from the horticultural sector in Andalusia (Spain) discussed the current situation and the main problems encountered in tomato and cucurbit crops affected by ToBRFV (Tomato Brown Rugose Fruit Virus), as well as ToLCNDV (Tomato leaf curl New Delhi Virus) and its whitefly vectors (e.g. *Bemisia tabaci*).

The workshop was organized by the local NKB, AA, with the participation of members of the Spanish network and the WPleaders to define the topics. This meeting was organized in the framework of the annual meeting of the project, promoting the discussion between project partners and local stakeholders.

During the workshop, two round tables in which participants discussed the topic of “Seeds and plant material” on the one hand, and “Farm management” on the other, were held.

Seeds and plant material round table conclusions

- **In the round table on “Seeds and plant material” it was concluded that the development time for breeding new commercial plant varieties with resistance to viruses is a limiting factor**, amounting to periods from 5 to 8 years for cucurbits and 8 to 10 years for tomatoes. These timelines could be significantly shortened with the introduction of gene-editing material produced by CRISPR/Cas9 (i.e. Clustered regularly interspaced palindromic repeats) technology, but this is currently not allowed for commercial purposes in the EU. The option of using public genebanks is a limited resource for private companies due to the delay in public research.

- **With regard to seed disinfection, the administrative requirement of declaring seeds as “virus-free seed” is considered to be a legal restriction which is not contributing to preventing the spread of viruses**. Moreover, there is no test on the market that can guarantee 100% that the seed is free of viruses. This finding, combined with the fact that infection can occur at any time during the process, makes it necessary to trace every step

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000570

and to homogenise the requirements in all regions. **With regard to the most widely used seed diagnosis methodologies**, the most important ones are bioassays or PCR and RT-qPCR for their accuracy; the Elisa test for its cost and the digital tools RT-qPCR and PCR for the time needed to obtain the results.

Findings of farm management round table

- **The round table on "farm management" addressed the management of ToLCNDV at farm level, thus establishing the state-of-the-art of whitefly control**. In this regard, the fact that there are hardly any known tomato varieties resistant to this vector and that *Bemisia tabaci* is the species with the highest incidence in Spain and Italy, compared to *Trialourodes vaporariorum*, which is most spread in Belgium, stands out. On the other hand, it is determined that crop management, both conventional and organic, has a greater influence on the distribution of the predominant genotype than climate change itself. Likewise, whitefly parasitoids and predators show a distinct preference for genotypes.

- **This round table also dealt with whitefly control methodologies**, identifying two of them as the most common in preventive control: natural enemies, with average effectiveness and farmer confidence, and crop management itself, valued with high effectiveness and average farmer confidence. **In terms of treatment methods**, the most supported option is the use of bio-based and/or residue-free pesticides, although their effectiveness is rated as medium-low, followed by chemical insecticides, which have high effectiveness and farmer confidence.

- **As far as ToBRFV is concerned, it is often identified through external laboratories**. The most common measures used for the management of ToBRFV on farms are disinfection, removal of affected plants, prohibition of external visits and application of hygienic measures for workers and tools after use. Within disinfection, solarisation in the case of

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soil and a series of measures such as the annual change of bags, crop rotation and disinfection with ozone for the substrate, stand out.

- **In terms of preventing the entry and spread of ToBRFV, a number of practices, considered highly effective by participants, are used**. These are hand, glove and tool washing, thorough initial cleaning of the farm, hygiene protocols for clothing and production, and awareness on the use of pesticides. **Concerning improving the on-farm control of the virus**, VIRTIGATION researchers recommended the implementation of a safe vaccine, resistant varieties and early monitoring systems, as well as increased sharing of measures to farmers, increased research and the implementation of the "quick wins" approach in the application of disinfection protocols, protection measures and management services. And on the farmer's side, soil hygiene measures, improving the farmer's own hygiene culture and improving knowledge about virus control, with clearer communication about disinfectants and resistant seeds, were supported.

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3.2. ToBRFV- Seminar: Connaissances, maitrise et Hygiene, organized in Agadir in January 2023 with the participation of Scientia Terrae, DCM CORP and Syngenta France

General information session giving an update on current ToBRFV knowledge, specifically focussed on practical information useful to around 200 growers.

A summary of the different points covered is given below:

- Our results show that spreading patterns within commercial greenhouses clearly indicate mechanical transmission as the primary transmission route for ToBRFV. Based on this pattern of spread, other transmission pathways including bumblebees, water, animals (such as rats and birds) and weeds, are of secondary importance.
- While water is a secondary transmission route for ToBRFV, the virus can still reach very high concentrations in the water. This allows us to use drain water as a monitoring tool/ early warning system to predict upcoming outbreaks.
- Transmission of ToBRFV in soil is possible and good crop practices should be applied to minimize risk of infection through this route (Dombrovsky et al., 2022). Steaming and solarization could provide possible solutions to deactivate ToBRFV in soil – this is being researched in the VIRTIGATION project.
- In a similar case study, we indicate that cross-contamination between neighbouring grower sites is a risk through the spread of dust. It of particular importance to keep windows closed when neighbouring farms are cleaning out their crops (i.e. when plant debris is burned outside).
- Although hand disinfection is of high importance for growers, not many options are available yet. A skimmed milk powder solution (5%) acts as a protectant against ToBRFV infection and can be used to disinfect hands. It is important to keep in mind, however, that milk powder solution does not deactivate the virus. Therefore, if used the milk powder solution should be refreshed regularly.

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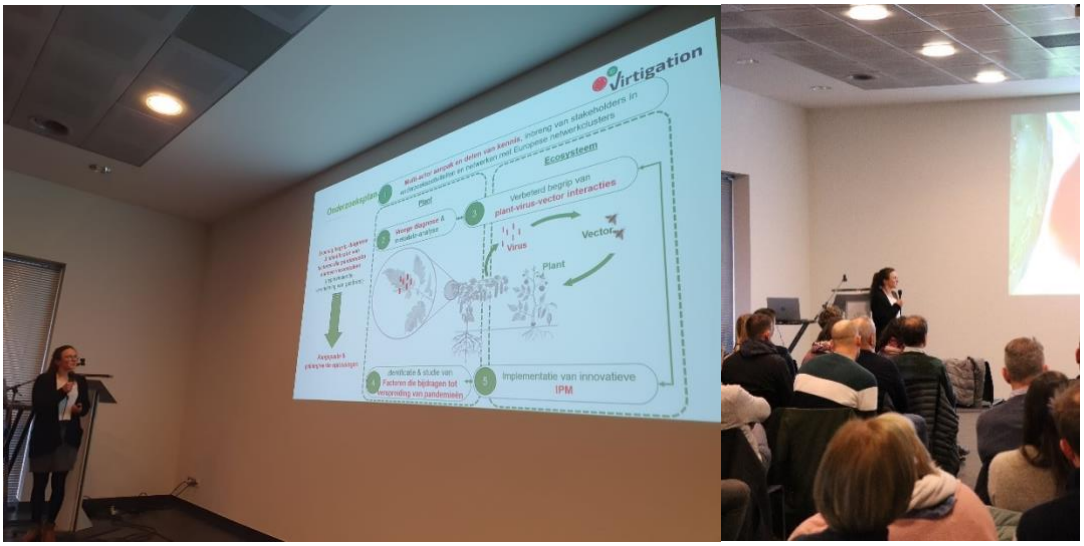
3.3. VIRTIGATION 1st multi-actor workshop in Belgium organized in January 2023 by PCH.

On 26 January 2023 Proefcentrum Hoogstraten organized a first Belgian workshop for VIRTIGATION. This workshop was part of a general meeting on IPM. During this meeting, several IPM topics were discussed, including plant viruses. In total, 112 stakeholders were present at the meeting, including farmers, agro-industry, agri-research centers, policy makers and partners from the VIRTIGATION project.

The aim of this first workshop was to introduce the VIRTIGATION project to the stakeholders, to extend the VIRTIGATION network and to present the first results. After the workshop, several participants commented that it is a very relevant project and that they are interested to hear more. However, no additional letters of intent were signed. We see two reasons for this: first, many participants already signed a letter of intent, and second, other stakeholders are looking for more practical results.

In conclusion we can say that the first VIRTIGATION workshop of VIRTIGATION was a success and many stakeholders joined. However, to reach more stakeholders in the future, more practical results should be shared.



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3.4. VIRTIGATION update in regular meetings from French community, organized by APREL and INRAE

The French community of growers is well stablished and organized thanks to the extension services from APREL, where regular meetings on growers requirements and problems are organized.

As the Virus pressure is actually low, French partners have dedicated the efforts to prevention and health monitoring with farmers, presenting VIRTIGATION program in the meetings and updating last advances.

Some of the actions pushed forward with French growers have been:

- Management plants for ToLCNDV, practice abstracts for growers and advisors how to face the problem
- Pest Risk Analysis on ToBRFV
- Co- Construction of field trials with stakeholders to test Zuchinni cultivars behaviour in farmers fields with natural infestation.

3.5. VIRTIGATION Netherlands stakeholders meeting organized in July 2022 by WU

A dedicated meeting organized in Bleiswijk (WUR Horticulture and Flowers Bulb) on July 2022 was organized by WU with Dutch growers. Although low participation, 5 stakeholders in total, discussion points offered valuable information on possible solution for virus problems and IPM measurements.

Main conclusions were the discussion about the importance of the hygiene methods and their high cots, and the implementation of readiness protocols. In addition, the transparency of communication of virosis detection is crucial to prevent and stop virus dissemination.

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3.6. VIRTIGATION field visits in India organized in January 2023 by NRI-UoG and UAS

Maruthi M N Gowda (NRI-UoG) visited the Virtigation H2020 project - save tomato & cucurbit from viruses partner at University of Agricultural Sciences, Bangalore to launch research on the role of seed transmission on viral disease spread.

In the South Indian state of Karnataka, they conducted a field visit for collecting fruits from virus-infected cucurbits for the research on the seed transmission of PlantViruses, especially ToLCNDV.

Then the team VIRTIGATION partner signed an MoU to intensify the research collaboration, and further enable a PhD student based there to support the project's research.

Finally two training activities were organized with 40 students for the Department of Plant Pathology in diagnosis of begomoviruses by PCR tests and management of plant virus disease epidemics.

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3.7. VIRTIGATION 1st multi-actor workshop in Germany organized in March 2023 by LNW

On the 23rd of March 2023, the VIRTIGATION National Partner for Germany, LNW, organised the first national multi-actor workshop. This workshop focused on the latest insights on ToBRFV for more than 50 local growers.

Since LNW is a state authority and not a federal authority, unfortunately, LNW can act only in the region of North Rhine-Westphalia and not nationwide. However, an attempt was made to reach interested growers in other regions of Germany and thus it was held as a hybrid event, i.e. participation was possible on-site and online. In particular, the tomato growers' circle from the Lower Rhine showed great interest in the event. In total 25 persons participated in the meeting, 17 live and 8 online. The event lasted almost 4 hours (13:30 - 17:30 pm).

The focus was on the general activities in the project, the activities of the LNW in the project as well as other important points around the ToBRFV management.

Programme:

1. "ToBRFV - Lessons learned from practice" (Dr. Elise Vogel, Scientia Terrae Research Institute, Sint-Katalijne-Waver/Belgium)
    a. Practical experiences on disinfection (hands, surfaces, mobile phones, ...)
    b. News on transmission and spread in greenhouses
    c. Drain water monitoring for early detection of ToBRFV

2. EU-project Virtigation: International cooperation against emerging viral diseases (Bianca Boehnke, Plant Protection Service, LWK NRW)
    a. Project presentation
    b. First results of decontamination methods of tobamovirus-infected substrates

3. Discussion, questions and miscellaneous

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After a short introduction by the organizer Mrs. Scholz-Döbelin, Dr. Elise Vogel started directly with her presentation. Due to her journey from Belgium and the strong interest in drainage water analysis, her presentation was put in the foreground.

Besides the drainage water analysis, other points were in focus of Elise Vogel´s presentation. First, Ms. Vogel gave a general introduction on ToBRFV symptoms and the distribution and situation in Belgium and the Netherlands. In 2022, a total of 18 sites in Belgium were affected. In the Netherlands, there were a total of 50 cases in 2022. Since 2019, the Netherlands has had a total of 67 affected producers.

The different detection methods were discussed and the sampling for ToBRFV analysis. The different detection methods (qPCR, ELISA, rapid tests) were explained and the accuracy of each method was discussed. For sampling, it is important to note that gloves must be changed after each sample. The highest viral load is in the head area of the plant and in the fruits, i.e. in the areas of the plant that are still growing. Older leaves in symptomatic plants may test negative, even when the younger leaves test positive. Samples should always be taken from the growing area.
A big question was the topic of disinfection. Although hand disinfection is of high importance for growers, not many options are available yet. In the discussion round, the modes of action of different disinfection methods such as milk powder solution, UV radiation or even classical disinfectants such as Fadex H+ and Menno Florades were discussed.

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In the next point of the presentation, results of experiments on the spread within a greenhouse were shown. The presented results showed that spreading patterns within commercial greenhouses clearly indicate mechanical transmission as the primary transmission route for ToBRFV. Based on this pattern of spread, other transmission pathways including bumblebees, water, animals (such as rats and birds) and weeds, are of secondary importance.

While water is a secondary transmission route for ToBRFV, the virus can still reach very high concentrations in the water. This allows us to use drain water as a monitoring tool/ early warning system to predict upcoming outbreaks. In the future, LWK's diagnostic laboratory will also offer drain water analysis.

In the second presentation, Mrs. Bianca Boehnke (LNW) presented the EU-project plan and also the first results of steaming. Before the emergence of ToBRFV in Germany, some tomato growers re-used the substrate mats from tomato production, e.g. for cucumbers or other crops. Since the emergence of ToBRFV, this kind of handling has been discontinued. Despite increasing resource scarcity and rising prices, the risk of carrying, multiplying and spreading potential viruses increased for the producers to a critical level. Thus, the substrate mats are disposed of after the harvest is completed.

On the other hand, we already have farms who suffered losses due to the Jordan virus or are at least very concerned about the prospect of an infection. They prophylactically steam all materials and also substrates before use to minimize a potential virus danger (and other harmful organisms). Through various scientific studies, it is already known that steaming at certain temperatures and steaming times can kill harmful microorganisms such as bacteria and fungus. This method has also been tested against viruses on different materials, but not yet on substrate mats (coconut substrate). Due to rising prices, increasing scarcity of resources and ever-increasing pest pressure, methods have to be developed for the future to save resources while working as hygienically as possible. Thus, the project Virtigation by LNW tries for the first time to decontaminate substrates contaminated with tobamovirus by steaming and to make them usable again. Based on different literature sources, conclusions have already been drawn as to what temperature and duration would probably have to be applied to coconut substrate. In the studies, the steaming duration 20min and 40 min at a steaming temperature of ≥ 90°C were used. In addition to classical contamination of substrate mats (i.e., by growth of tobamovirus-infected tomato), substrate mats were additionally prepared with infected root mass (A; 350g per mat) and infected leaf mass (B; 500g per mat). To verify if the steaming was successful, a bioassay must be performed. That is, healthy tomatoes must be planted again in the steamed substrate mats and regularly checked by examining the tomatoes to see if they remain healthy. If this is the case, the steaming is successful. If infected tomatoes appear, the steaming was unfortunately not successful.

Parallel to the steamed mats, positive controls were also kept, i.e. tobamovirus-contaminated substrate mats without steaming. Tomatoes in these non-steamed virus-contaminated substrate

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mats showed clear symptoms already after 3 weeks. At week 4, the first ELISA and at week 10, the second ELISA were performed on the tomatoes. The results of the first ELISA were positive, i.e., tomatoes in the steamed mats were virus-free, while tomatoes in the non-steamed mats were clearly positive. Unfortunately, the results of the second ELISA were not yet available at the time of presentation, but the tomatoes in the steamed mats were still asymptomatic at that time. After completion of the bioassay with the tomatoes in the substrate mats, a bioassay with the substrate will also be performed again on the very sensitive tobacco cultivar Nicotiana tabacum 'Xanthi nc` for safety.

The growers showed great interest in the results on the steaming. Some growers already have their material prophylactically steamed for safety, as mentioned before. Since it is often not entirely clear where the initial infection comes from, the plant managers want to minimize all potential sources of danger as far as possible. Up to now, they have worked with the highest possible steaming temperature (≥ 100°C) and with the longest possible steaming time (min. 24 h). But here, too, it must be noted that not all materials can withstand such high steaming temperatures, and material resistance often suffers, too. It would give the producer a great deal of certainty to know whether the steaming additionally shows an effect against viruses at lower temperatures and shorter steaming times. If this method is confirmed, other producers will also be interested in using this method.

Conclusions

It was a very successful event. The producers showed great interest in the topic ToBRFV. The main questions are still open

1. How does the virus get into the crop?
2. How can I protect my crop as much as possible (hygiene)?
3. What is the best detection method? (There is a strong need for a method that can specifically detect active virus particles).
4. How can I optimally disinfect the production facilities after an emergence? Especially the areas that cannot be cleaned with e.g. Menno Florades or Fadex H+ (such as recreation rooms and sleeping quarters of the harvest workers, walkways on the property). And how can I prove that the production facility is now really virus-free (so far missing methods to distinguish active from inactive virus particles).

The producers showed a lot of interest in the research into steaming. When it is clear that this method really works, some producers will switch back to steaming to save costs.

Furthermore, a report on the event and especially on the drainage water analysis has been produced in the Plant Protection Special Information Service of the LWK.

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3 QUESTIONNAIRE TO FARMERS

As planned in the project proposal, a structured questionnaire has been produced jointly by partners 8 – UNICT (Italy), 14 – PCH (Belgium), 15 – APREL (France) and 20 – AA (Spain); in addition to these partners, explicitly planned in the proposal, also partner 3 – TECNOVA has been involved, to help partner 20 – AA in performing the investigation activity in Spain.

The main aim of the questionnaire has been to **gather useful information on the state of whiteflies control** (with special reference to *Bemisia tabaci*) and whiteflies related virus problems in different cropping systems with both conventional methods (chemicals used, doses, frequency of application, etc.) and biorational ones (natural enemies used and their methods of rearing and releasing, bioinsecticides, physical and mechanical control methods used, etc.).

Per each interviewed entity, specific questions have been applied aimed at gathering information on the farm owner (age, sex, educational qualification), farm organization (surface, cropping system, horticultural productions realized), whiteflies management applied on the main crop cultivated in the farm (preventive / cultural strategies, such as healthy plant material, weeds control, etc.; structural / physical / mechanical strategies, such as mulches, nets, traps, etc.; biological control, such as predators, parasitoids or microbes; chemical control, in terms of product categories used and number of treatments applied).

Once defined (in English), the questionnaire has been translated in Dutch, French, Italian and Spanish and distributed online to a large number of farmers, food producers, technicians, horticultural products marketing companies etc. in Belgium, France, Italy and Spain.

Altogether, 208 responses have been received in the four countries (Belgium: 43; France: 36; Italy: 47; Spain: 82). This big set of data gives a wide overview on the main strategies which are presently applied for whitefly control in different European environments, from south to central Europe, and diverse cultural context. Results have been already analysed per single country, giving some basic and general feedback on:

i) the still low percentage of totally organic farms, with integrated production prevailing;
ii) the most widely applied natural enemies, with *Macrolophus pygmaeus* and Nesidiocoris tenuis being the main predators used and *Encarsia formosa* and *Eretmocerus eremicus* the most diffused parasitoids; iii) how microbial products are being increasingly used in both organic and integrated cropping systems; iv) how chemical control is still the prevalent control method applied, although with new products increasingly used (such essential oils, polymers, etc.).

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In the comming months, the questionnaire will be extended to additional countries, including farmers from other European (Germany, The Netherlands) and non-European (Israel, Morocco) countries as well. The final results obtained will be available by a paper proposed for publication to a scientific journal and dissemination activities to be organized through local networks.

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