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

Deliverable D4.3
Reports on the role of different whitefly species and weed hosts on viral disease epidemics

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Reports on the role of different whitefly species and weed hosts on viral disease epidemics (M36, R, PU)

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LIST OF ABBREVIATIONS

CSIC - The Spanish National Research Council
ELISA - Enzyme-linked immunosorbent assay
INRAE - National Research Institute for Agriculture, Food and the Environment
MEAM1 – Middle East Asis Minor 1
MED - Mediterranean
PepMV-IL - Pepino mosaic virus-IL strain
qRT-PCR - quantitative real-time polymerase chain reaction
RCA – Rolling circle amplification
RFLP -Restriction fragment length polymorphism
SSA2 – Sub-Saharan African 2
ToBRFV – Tomato brown rugose fruit virus
ToLCBV - Tomato leaf curl Bangalore virus
ToLCNDV-ES Tomato leaf curl New Delhi virus – ES strain

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1 PUBLISHABLE SUMMARY

In this task, we carried out diverse sets of experiments to investigate the potential spread of the two emerging plant viruses in Europe: tomato brown fruit rugose virus (ToBRFV) tomato leaf curl New Delhi virus (ToLCNDV). First, we investigated the competence of different whitefly species transmitting ToLCNDV using the native Indian whiteflies with the native Indian virus and found that the native whitefly species transmitted the virus better than the invasive species. This supported the co-evolution hypothesis where the viruses, vectors and their host plants are better adapted to each other. Thus, strategies for controlling ToLCNDV should also include controlling the native Indian whiteflies. Similar experiments were carried out in Spain where the native SSA2 was shown to transmit ToLCNDV equally efficiently as the invasive MED species. These slightly contrasting results could be the wide-spread establishment of the MED in Europe now for more than two decades while the MED or MEAM1 are not widely established in India. Controlling both native and invasive species is essential for controlling ToLCNDV in Europe. Moreover, we also investigated the transmission potential of ToLCNDV by the glasshouse whitefly, *Trialeurodes vaporariorum*, but luckily this species does not transmit ToLCNDV.

In a second set of experiments, we investigated the alternative host plants for ToLCNDV and ToBRFV. We found a very few alternative host plants for both viruses; Three plants each were infected by each virus. Ecballium, bryony and jimsonweed were confirmed to be alternative hosts for ToLCNDV although the former does not contribute to disease spread as the virus was not transmissible from Ecballium to zucchini. *Solanum nigrum*, *S. elaeagnifolium* and *S. rostratum* were found to be the alternative hosts of ToBRFV. All the alternative hosts should be removed from tomato and cucurbit fields for minimizing the spread of the viruses into the main crop. The implications of our findings for disease control are presented in this report.

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2 INTRODUCTION

The whitefly *Bemisia tabaci* is a global pest causing major diseases of crop plants in the Mediterranean, tropical and sub-tropical regions of the world including the protected environments in temperate regions. It was known as a secondary pest until the 1980s (Byrne et al., 1990) but the host and geographical range of the pest increased dramatically in the last four decades and thus attained a primary pest status. *B. tabaci* is a complex species (Wang et al., 2024) with several populations exhibiting distinct biological and behavioural properties. It has a wide host range, feeding on at least 600 plant species causing huge economic losses to growers. It causes damage to the plants directly by feeding and indirectly by transmitting plant viruses (Byrne et al., 1990). By far the most serious damage is caused by transmitting many economically important plant viruses (currently about 500 species) belonging to five virus genera: *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus* and *Torradovirus* from different virus families (Navas-Castillo et al., 2011; Fiallo-Olivé and Navas-Castillo, 2023). Over 400 species of begomoviruses are exclusively transmitted by *B. tabaci* adults which is the largest group vectored by *B. tabaci*. The global increase in begomovirus diseases during the last three decades has been partly due to the aggressive spread of two *B. tabaci* biotypes, called B (MEAM1 species) and Q (MED species), which are also capable of transmitting viruses efficiently in new areas. However, the invasive B and Q biotypes have not established themselves in India as they have not out competed the native local populations. In contrast, both B and Q were introduced to Europe and Q has established itself and been the predominant population for more two decades, capable of transmitting all begomoviruses present in Europe. The combination of the presence of Q biotype, the fast spreading nature of ToLCNDV and the susceptibility of the varieties grown is responsible for the current outbreak of the cucurbit leaf curl disease in the Mediterranean region. While a different species, the greenhouse whitefly house *Trialeurodes vaporariorum* was shown to transmit cucurbit infecting ToLCNDV strain in India, which was raising the possibility of increased vector domain for ToLCNDV. To investigate all these possibilities and to determine the host range of ToLCNDV and one other fast spreading virus ToBRFV in Europe, we carried out several transmission and host range experiments in Virtigation. The implications of these results are discussed in this report.

3 ESTIMATING THE COMPETENCE OF WHITEFLIES ON VIRAL DISEASE SPREAD

Such is the diversity of whiteflies in India, it was once considered the centre of origin of whiteflies. Several biotypes of *B. tabaci* are therefore exist in India but their competence to spread viruses was not known. To investigate the vector competence of Indian whiteflies, seven different *B. tabaci* biotypes/ species (MED, MEAM1, Asia I, Asia II-5, Asia II-7, Indian cassava and an out group from Nigeria) were investigated to their ability to transmit cucurbit-infecting ToLCNDV, which is endemic to India. It was anticipated that the understanding the transmission of the native virus from India will help us understand the introduced virus in the Europe. In a separate experiment, transmission the virus was also confirmed by wedge-grafting of diseased scion on to healthy tomato plants

For the transmission experiments, the whiteflies collected previously and maintained at NRI-UK in this project were used. About 500 adults were collected from the colonies and released on to virus-infected plants for an acquisition access period of 24 hours in lock-lock pots. Ten viruliferous whiteflies were then collected from diseased plants and released onto two weeks old young tomato plants individually

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of the variety Money maker inside a cylindrical cage for virus inoculation (24 hours inoculation access period). Whiteflies were removed subsequently and the plants were incubated for 21 days to express symptoms in the quarantine laboratories. Our results showed that the whiteflies -originated from India transmitted ToLCNDV better than the invasive MED (Q biotype) and MEAM1 (B-biotype) or the outgroup from Africa (Nigeria). Our results thus supported the well-established hypothesis of the co-evolution of viruses, vectors and their host plants from a single geographical region. Among the Indian whiteflies, Asia II-7 transmitted with 100% efficiency while the Aisa I and Asia-II 5 with 62.5 and 50% efficiency. The transmission experiment was repeated on another Indian strain, tomato leaf curl Bangalore virus (ToLCBV) and its transmission was also confirmed to be 100% by the Asia II-7 species. In another experiment, 100% transmission was achieved for both ToLCBV and ToLCNDV by wedge-grafting of diseased tomato scion on to healthy tomato plants var. Money maker.

Implications: Local whiteflies transmitted ToLCNDV better than the invasive species, which supported the co-evolution theory of viruses, vectors and their host plants. Strategies for controlling viral diseases should not neglect the local whitefly species, which spread the viruses more efficiently.

4 ESTIMATING THE COMPETENCE OF EUROPEAN WHITEFLIES ON TOLCNDV-ES SPREAD

In similar experiments to estimate the vector competence of the ToLCNDV-ES, the Spanish strain, the native whitefly species MED and SSA2 were tested for their vector competence in Spain.

- The pure colonies of *B. tabaci* species MED and SSA2 were established at CSIC-Spain to carry out the transmission experiments.
- Transmission of an isolate of ToLCNDV-ES from zucchini to *Ecballium elaterium* plants (a wild cucurbit known to be an alternative host for ToLCNDV) and *vice versa* was assessed in replicate experiments using *B. tabaci* MED. The results showed that transmission between both plant hosts was inefficient, suggesting that *E. elaterium* is a dead-end host for this virus. *E. elaterium* is therefore unlikely contribute to disease spread in field conditions. In comparison about 50% transmission efficiency was achieved between zucchini plants.
- Transmission of ToLCNDV-ES was also assayed between zucchini plants using *B. tabaci* SSA2 as a vector. This whitefly species was able to transmit the virus with an efficiency similar to that attained with the control *B. tabaci* MED, the most prevalent species in the Mediterranean basin. Both invasive and native species therefore spread ToLCNDV equally efficiently in Spain.

Implications: The widely established MED and the locally found SSA2 can both transmit ToLCNDV equally efficiently. Controlling them both is critical for minimizing virus spread.

5 ASSESSMENT OF TOLCNDV TRANSMISSION BETWEEN ZUCCHINI PLANTS BY *TRIALEURODES VAPORARIORUM*

In these experiments, we investigated the transmission potential of ToLCNDV by the glasshouse whitefly *Trialeurodes vaporariorum.*

- The pure colony of *T. vaporariorum* were established both at CSIC-Spain and INRAE-France for transmission experiments.

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- In Spain, transmission experiments between zucchini plants using ToLCNDV-ES-agroinoculated plants as source of virus and *T. vaporariorum* adults as vectors were carried out in replicate experiments including *B. tabaci* MED as a positive control. No transmission was obtained with *T. vaporariorum* whereas *B. tabaci* MED was able to transmit the virus in the same experimental conditions to more than 50% of the zucchini plants.
- Similarly, both the French and Spanish (used as reference) isolates of ToLCNDV were transmitted by *Bemisia tabaci* MED biotype by INRAE-France but not by *Trialeurodes vaporariorum*, confirmed the results obtained by Spanish.

Implications: The glasshouse whitefly *T. vaporariorum* is not involved in ToLCNDV transmission so we do not need to consider this as a threat to spread the virus although it can be a direct pest by itself feeding on tomato and cucurbits.

6 DETERMINING THE HOST RANGE OF TOLCNDV-ES EUROPEAN STRAIN

We have carried out experiments in both Spain and France to investigate the alternative hosts of ToLCNDV in natural conditions.

- CSIC carried out a survey in southern Spain (provinces of Málaga and Cádiz) and identified *E elaterium* plants infected with ToLCNDV-ES. Survey in the province of Málaga was later extended to many species of the families Amaranthaceae, Apiaceae, Apocynaceae, Boraginaceae, Cannabaceae, Compositae, Convolvulaceae, Euphorbiaciae, Geraniaceae, Lamiaceae, Malvaceae, Nyctaginaceae, Polygonaceae, Scrophulariaceae, Solanaceae, Urticaceae, Verbenaceae and Vitaceae. In total, 400 samples belonging to 30 species were analyzed for the presence of ToLCNDV-ES by molecular hybridization and RCA/RFLP. The only plant species found to be infected to date was *E. elaterium* and percentage of infection on different plant species ranged from 0 to 40%. The plant species tested included *Achyrantes aspera, Amaranthus sp., Anaciclus radiatus, Atrichea viscosa, Bidens pilosa, Celtis australis, Centaurea sp., Convolvulus sp., Datura stramonium, Ecballium elaterium, Euphorbia sp., Geranium sp., Heliotropium europaeum, Ipomoea indica, Lantana camara, Malva sylvestris, Mentha suaveolens, Mirabilis jalapa, Nerium oleander, Nicotiana glauca, Parietaria officinalis, Plantago major, Ricinus communis, Rubia peregrina, Rumex sp., Solanum nigrum, Sonchus sp.,* and *Verbascum sinuatum*.
- Several ToLCNDV-ES isolates obtained from naturally infected *E. elaterium* plants were molecularly identified, cloned and sequenced. One of these isolates was completely sequenced and used for making agroinfectious clones of DNA-A and DNA-B viral genomic components by CSIC-Spain.
- In addition, weed and cultivated plants common in South-eastern France have been tested by mechanical inoculation for ToLCNDV infection. Our results showed that white bryony (*Bryonia dioica*), a perennial cucurbit weed common in South-eastern France, is susceptible to ToLCNDV and could constitute a reservoir. Preliminary transmission experiments by *B. tabaci* MED in controlled conditions showed that whitefly transmission between bryony and melon is possible. Surveys close to infected cucurbit crops in natural conditions revealed ToLCNDV infection in Ecballium, bryony and jimsonweed (*Datura stramonium*).

Implications: In addition to cucurbits, which is high susceptible, we have identified at least three alternative hosts of ToLCNDV in Spain and France: Ecballium, bryony and jimsonweed. While the

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virus could not be transmitted by whiteflies from Ecballium and thus does not contribute to disease spread immediately but the virus was transmissible from the latter two species and thus they can be reservoir of the virus during the off season. Bryony and jimsonweed should therefore be removed to prevent them from providing the initial virus inoculum for the new crop of cucurbits while Ecballium should also be removed for preventing the development of a new strain of virus in this reservoir host which might regain its transmission abilities in future climatic conditions.

7 DETERMINING THE HOST RANGE OF TOBRFV

To investigate the potential of wild weeds of another fast spreading virus, ToBRFV, we focused on *solanaceae* plants that commonly occur in Israel.

- We have established propagation methods by sap-mechanical inoculations for studying susceptibility and host potential for ToBRFV and an endemic PepMV-IL as mixed infections of these two can be found commonly on tomatoes by synergism.
- We have calibrated a panel of methods for ToBRFV and PepMV-IL detections by: qRT-PCR, Western blots, ELISA and biological assays.
- Various invasive weeds were tested as hosts of ToBRFV. Three plant species belonging to the *Solanaceae* family were proved as hosts of ToBRFV: *Solanum nigrum*, *S. elaeagnifolium* and *S. rostratum*. Two out of the three species: *S. nigrum* and *S. rostratum* have shown synergism between ToBRFV and the mild PepMV-IL exhibiting a high titter of PepMV-IL and *S. rostratum* plants have shown highly severe symptoms upon mixed infections.

Implications: Similar to ToLCNDV, ToBRFV was also found to have three potential reservoirs: *Solanum nigrum*, *S. elaeagnifolium* and *S. rostratum*. These should be removed to prevent the build of ToBRFV inoculum in tomato fields and glasshouses.

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ANNEX 1: Some pictures from the experiments conducted in this task.

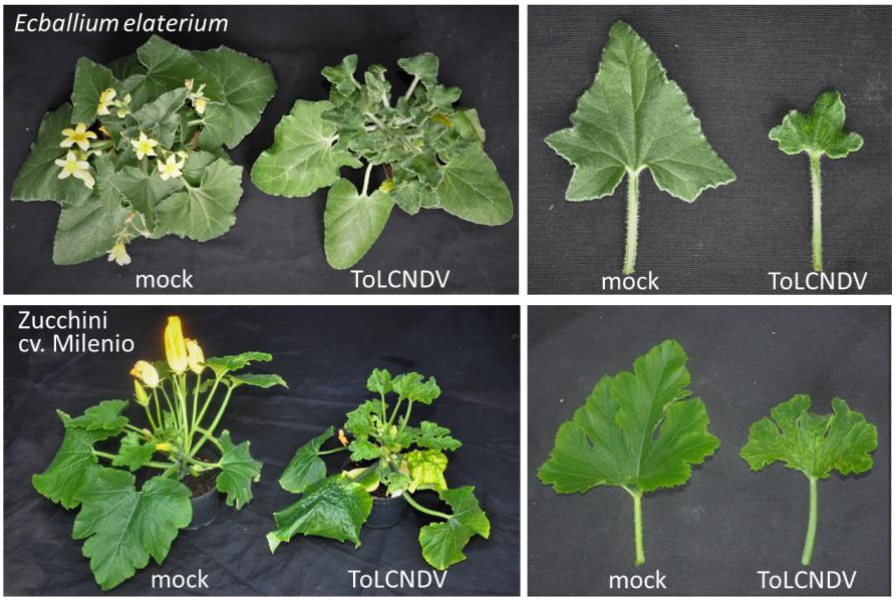

Figure 1. Zucchini and *E. elaterium* showing plants and leaves showing ToLCNDV symptoms are shown. The wild cucumber *Ecbalium* plant is the only known alternative host found infected with ToLCNDV in Spain, although two additional hosts bryony and jimsonweed were also found in France.

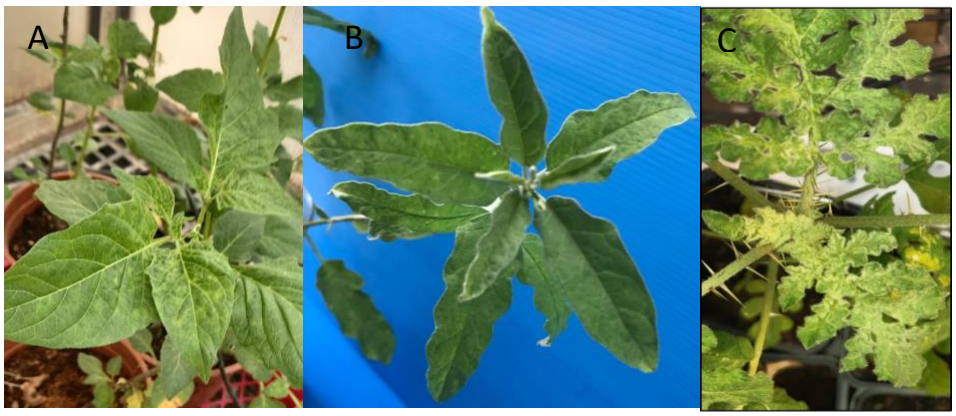

Figure 2. *Solanum nigrum* (A), *S. elaeagnifolium* (B) and *S. rostratum* (C) are the only three known wild hosts of ToBRFV in Israel in the Solanaceae family.

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