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

Deliverable 4.1
Report on the effects of climate change on the current and future spread of viral diseases in Europe

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Report on the effects of climate change on the current and future spread of viral diseases in Europe (M42, R, PU)

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LIST OF ABBREVIATIONS

LIST: Luxembourg Institute of Science and Technology
UniCT: University of Catania
CSIC: Spanish National Research Council
Bemisia tabaci MED: Mediterranean species (formerly Q biotype) of the *Bemisia tabaci* complex
BCAs: Biocontrol Agents
ToLCNDV: Tomato leaf curl New Delhi virus
ToLCNDV-ES: Tomato leaf curl New Delhi virus, Spanish strain
AAP: Acquisition Access Period
IAP: Inoculation Access Period
dpa: days post-acquisition
dpi: days post-inoculation
ddPCR: droplet digital PCR
qPCR: quantitative PCR
SEV: severe phenotype
REC: "recovery" phenotype

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1 PUBLISHABLE SUMMARY

In the context of the VIRTIGATION project, the LIST group conducted comprehensive studies to evaluate how projected future climate conditions may affect the *Bemisia tabaci* (MED) – ToLCNDV – zucchini pathosystem. The experimental design included two climate scenarios - current and future climatic conditions (based on RCP 8.5 projections for 2061-2070) - applied across multiple experiments to assess the impacts of environmental changes on *B. tabaci*, their biocontrol agents, and the transmission of ToLCNDV. Moreover, INRAE estimated the effect of temperature on the symptomatology and accumulation of ToLCNDV, and the durability of resistance against it under this abiotic stress.

The first set of experiments focused on the effects of climate change on two whitefly biocontrol agents, *Encarsia formosa* and *Eretmocerus eremicus*, aiming to understand how future climate conditions might alter natural pest control strategies. Results showed that the survival of *E. formosa* and *E. eremicus* adults was negatively impacted under future climate conditions, whereas nymphal development accelerated, and reproductive rates increased. These findings suggest that biocontrol strategies may need to be adapted or supplemented in future climates to ensure effective pest management.

The second set of experiments investigated the influence of climate on ToLCNDV acquisition and inoculation mediated by *B. tabaci* MED. Under future climate conditions, the results revealed a higher efficiency of ToLCNDV acquisition and inoculation by the *B. tabaci* MED. This increase in virus acquisition and inoculation efficiency under warmer conditions suggests that *B. tabaci* MED could become more effective at spreading the virus, potentially leading to more severe ToLCNDV outbreaks in future growing seasons.

As part of these experiments, the LIST group developed and optimized a new method for the absolute quantification of ToLCNDV using droplet digital PCR (ddPCR). Preliminary results showed the ability to detect both ToLCNDV DNA components and plant genomic DNA in the same run, offering a valuable tool for monitoring viral loads in infected plants. This method will support future studies by providing precise quantification of plant virus spread, enhancing efforts to track and manage viral outbreaks effectively.

The results from these experiments have significant implications for the future management of agricultural pests and viruses. The already reported accelerated development and increased reproductive capacity of *B. tabaci* under future climate conditions may result in more frequent pest cycles and larger populations, amplifying the risk of ToLCNDV transmission. Considering these negative effects together with the reduced survival of biocontrol agents, there may be a need of adaptations in the biocontrol strategy, such as more frequent augmentative releases of beneficial insects.

The enhanced efficiency of *B. tabaci* in acquiring and inoculating ToLCNDV under projected climate scenarios underscores the urgency of researching climate-resilient crop protection strategies. Precise tools like ddPCR will be crucial for monitoring viral loads and spread, empowering farmers to implement more targeted and effective management measures.

Interestingly, INRAE results showed no association with more severe viral symptoms or enhanced ToLCNDV multiplication in the higher temperature regime, partially in contrast to LIST results.

These results are indicating how essential is to integrate climate change considerations into plant protection research, for preparing agriculture to meet future challenges. Proactively addressing the effects of climate change on *B. tabaci*, its biocontrol agents, and ToLCNDV transmission will be critical to safeguarding food security and promoting sustainable crop production in an evolving climate.

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2 INTRODUCTION

Bemisia tabaci, a whitefly, is a globally distributed agricultural pest with a broad host range, including many economically important crops (Kanakala and Ghanim, 2019). It is in fact a complex of cryptic species, each varying in its biology, behavior, and capacity to transmit plant viruses (EFSA Panel on Plant Health (PLH), 2013). In particular, the latter plays a crucial role, as they can transmit over 500 viruses (Fiallo-Olivé et al., 2020). Among them, begomoviruses are one of the most representative groups, and recently the tomato leaf curl New Delhi virus (ToLCNDV) has caused a disease outbreak on cucurbits in Europe (European and Mediterranean Plant Protection Organization, 2024). The high reproductive rate of the *B. tabaci*, its polyphagy, and the ability to develop resistance to insecticides make it a significant threat to global food security (Horowitz et al., 2020).

The MED species of the *B. tabaci* complex, previously known as the Q biotype, is one of the most invasive and problematic members of the *B. tabaci* complex. It is characterized by high resistance to chemical control (Horowitz et al., 2020) and exceptional efficiency in transmitting plant viruses (Fiallo-Olivé et al., 2020). *B. tabaci* MED populations thrive in warm climates, making them especially responsive to environmental changes. Their adaptability allows them to colonize diverse regions and agricultural systems, compounding their threat as a pest and vector of plant pathogens.

Integrating climate change considerations into *B. tabaci* research is vital for addressing future challenges. Rising temperatures and shifting ecosystems under climate change scenarios are expected to influence the biology, distribution, and virus transmission efficiency of *B. tabaci*. Research that models pest and virus behavior under future climates enables proactive pest management strategies, mitigates potential crop losses, and strengthens global agricultural resilience. Addressing climate change in *B. tabaci* studies is not merely beneficial but essential for achieving sustainable crop protection in an increasingly unpredictable environment.

With this goal in mind, Task 4.3 (“Investigate the role of climate change on begomovirus biology and spread”) focused on assessing the impacts of projected future climates on the *B. tabaci* – ToLCNDV – zucchini pathosystem and the fitness of the whitefly antagonists.

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3 INFLUENCE OF CLIMATE ON BEMISIA TABACI AND ITS ANTAGONISTS

The impact of climate change on *Bemisia tabaci* MED, (originally collected in Sicily, provided by Carmelo Rapisarda, UniCT, Italy) has been recently assessed by the LIST group, revealing that the projected mid-future (2061-2070) Central-European climate (Figure *1*) would accelerate whitefly nymphal development, reduce adult longevity while increasing its prolificacy (Milenovic et al., 2023a). For a full overview on the climate projections refer to Milenovic et al., 2023a.

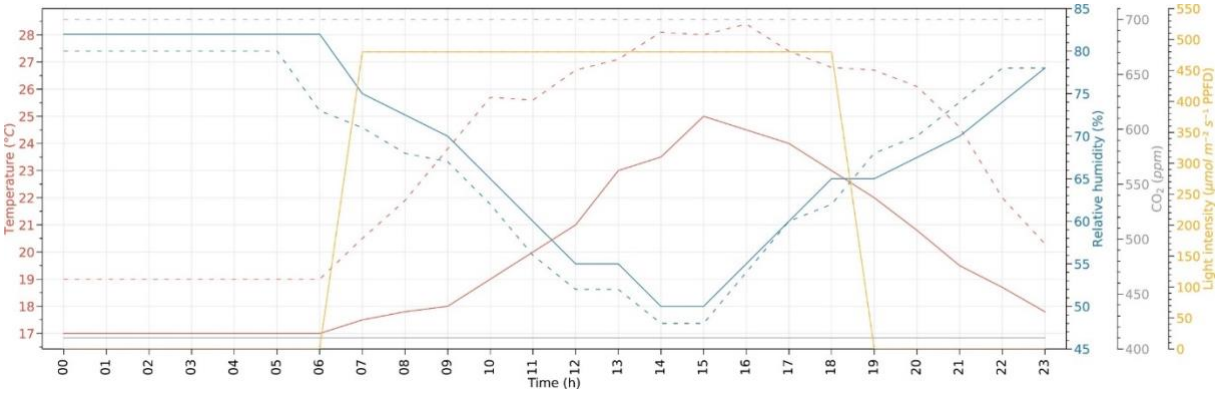

Figure 1. Daily courses of air temperature, relative humidity, CO2 concentration, and light intensity used to drive climate chambers for present and future conditions. Solid and dashed lines represent the data for present and future conditions, respectively. The mean parameters for the two climates were as follows. *Present*: temperature (T) = 19.8°C, relative humidity (RH) = 69.2%, CO2 concentration = constant 410 ppm; *future*: T = 23.4°C, RH = 67.5%, CO2 concentration = constant 700 ppm. From Milenovic et al., 2023a.

As part of the VIRTIGATION project, we assessed how future climate will affect two whitefly biocontrol agents (BCAs), *Encarsia formosa* and *Eretmocerus eremicus* (Milenovic et al., 2023b-c). Our results showed a reduction in their survival time (~40%, Figure *2*, Figure *3*), an acceleration in the nymphal development (by ~9 days, Figure *2*), and an increase in the female prolificacy (~15-fold higher parasitization rate).

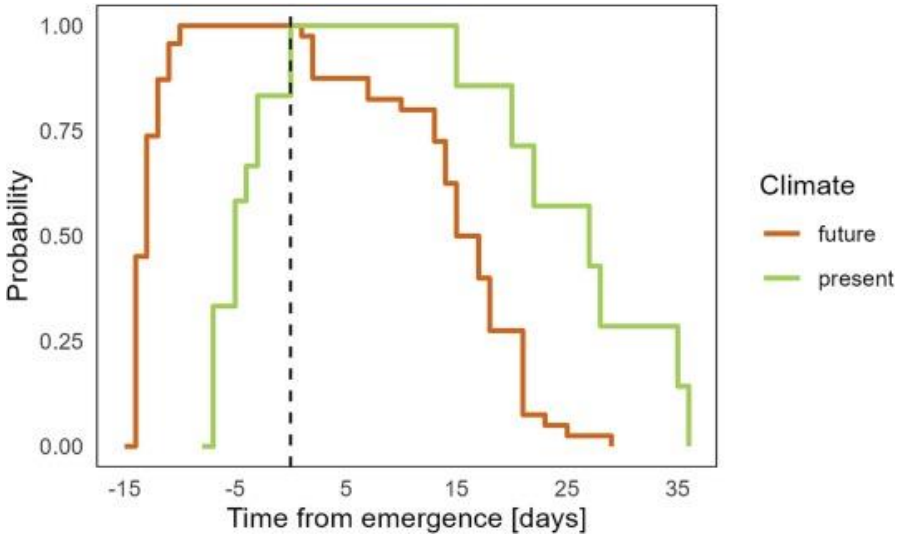

Figure 2. Joint development curves (left of the dashed line) and longevity curves (right of the dashed line) of F1 adult *Encarsia formosa* under present (2006–2015, green) and future (2061–2070, red) climate conditions. Area under the curve is 28.6, and 27.3 for the present and future curve, respectively. From Milenovic et al., 2023b.

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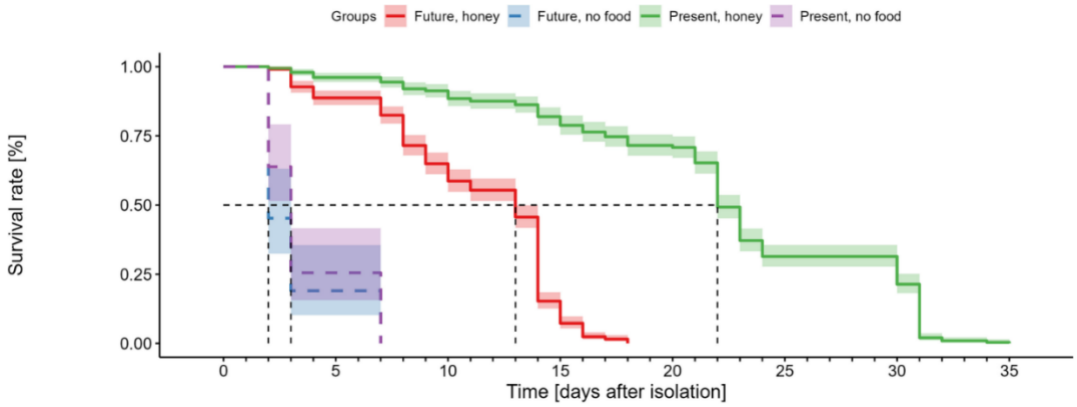

Figure 3. Survival curves (Kaplan-Meier estimates) for adult *Eretmocerus eremicus* under present (2006–2015) and future climate conditions (2061–2070). Groups are divided based on climate (present vs future) and diet (honey vs no-food control). From Milenovic et al., 2023c.

Overall, these changes may reflect in the future with increased costs for agricultural growers: a faster development time of whiteflies potentially means more cycles over the growing season and a higher number of individuals. The increase may be counteracted by the increased prolificacy of the biocontrol agents, but the lowered survival may reflect in the need of an increased BCAs release over time.

4 INFLUENCE OF CLIMATE ON TOLCNDV TRANSMISSION

4.1 Influence of climate on ToLCNDV acquisition

In order to assess the influence of climate on the acquisition of the ToLCNDV by *B. tabaci* (MED), LIST set up an experimental design, briefly reported below.

The two above-mentioned climate scenarios (Figure *1*, Milenovic et al., 2023a) were applied. ToLCNDV-infected plants (Figure 6) were obtained by agroinoculating *Agrobacterium tumefaciens* (C58C1, GoldBio) transformed with ToLCNDV-ES infectious clones (provided by Jesús Navas-Castillo and Elvira Fiallo-Olivé, CSIC, Spain). Before starting the experiments, the infectivity was assessed by PCR (Figure 7, Ruiz et al., 2015). Adult whiteflies were collected with a mouth aspirator, shortly anesthetized on ice, and isolated in clip-cages on leaves of ToLCNDV-infected plants. Three different Acquisition Access Periods (AAPs) were tested - 2 hours (AAP1), 24 hours (AAP2), and 48 hours (AAP3). At the end of each AAP, the whiteflies were collected and isolated with clip-cages on healthy zucchini plants (*Cucurbita pepo* var. Amorgos, Syngenta) at the first true-leaf stage (Figure 8), five whiteflies per plant. A constant Inoculation Access Period (IAP) of 48 hours followed. At the end of the IAP, the status of the whiteflies was recorded (alive, dead), and the alive specimens were collected and frozen at -80°C. The whole experiment was replicated twice (n = 54; Figure 4).

This design allowed for a comprehensive evaluation of how climate changes might alter virus acquisition efficiency over varying exposure durations.

The collected results (Figure 4) indicated a pronounced effect of the future climate scenario on ToLCNDV acquisition efficiency by the whitefly vector, suggesting that the projected climatic changes could significantly enhance the virus transmission dynamics.

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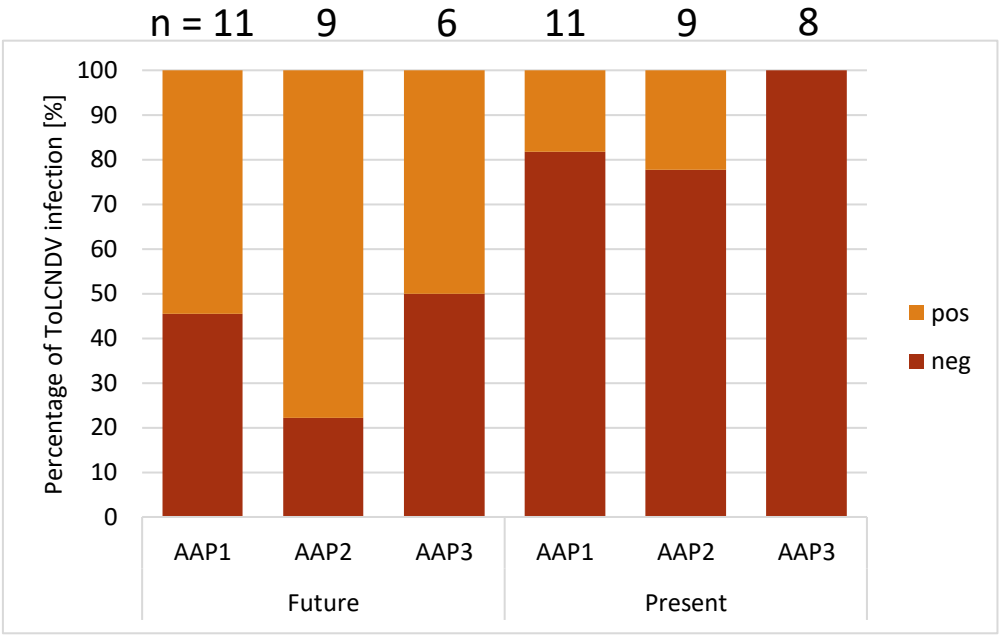

Figure 4. Stacked bar plot of the ToLCNDV acquisition experiments. x-axis: present (2006-2015) vs future climate (2061-2070); AAP1: 2 hours, AAP2: 24 hours, AAP3: 48 hours. y-axis: percentage of ToLCNDV infection. Red bars are indicating negative plants after four-weeks latency after the inoculation, while orange bars are indicating positive plants. The total number of plants exposed to the different combination of experimental conditions is reported above the graph (sum of the two experimental replicates).

4.2 Influence of climate on ToLCNDV inoculation

Similarly to the acquisition experiment, LIST set up the following experimental design to assess the influence of climate on the inoculation of ToLCNDV by *B. tabaci* MED.

The same climate scenarios were applied (Figure *1*, Milenovic et al., 2023a), and the already mentioned protocol for obtaining ToLCNDV-infected plants and infectious insects was followed. Adult *B. tabaci* were isolated on ToLCNDV-infected zucchini plants for a constant AAP of 48 hours. After the AAP, five *B. tabaci* adults were collected and isolated by clip-cages on healthy zucchini plants (*Cucurbita pepo* cv. Amorgos, Syngenta) at the first true-leaf stage (Figure 8). The plants were separated in three groups, exposed to different Inoculation Access Periods (IAPs) by the infectious whiteflies: 2 hours (IAP1), 24 hours (IAP2), and 48 hours (IAP3). At the end of the IAP, the status of the whiteflies was recorded (alive, dead), and the alive specimens were collected and frozen at -80°C. The whole experiment was replicated twice (n = 62; Figure 5).

Results indicated that *B. tabaci* MED exhibited increased ToLCNDV inoculation efficiency under future climate conditions, highlighting potential implications for virus transmission dynamics in a warming climate.

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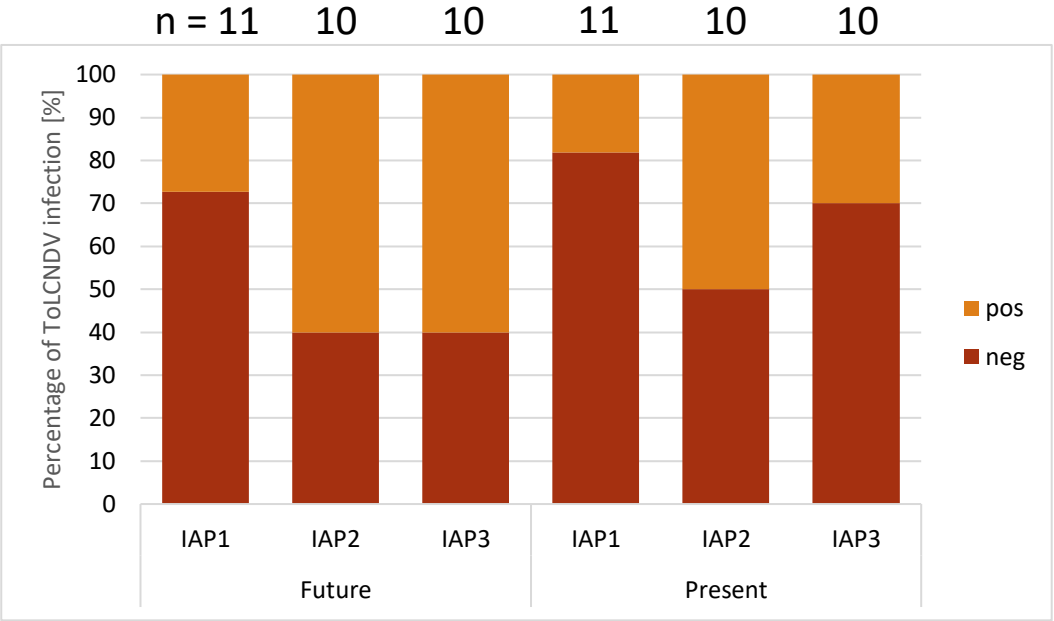

Figure 5. Results of the ToLCNDV inoculation experiments. x-axis: present (2006-2015) vs future climate (2061-2070); IAP1: 2 hours, IAP2: 24 hours, IAP3: 48 hours. y-axis: percentage of ToLCNDV infection. Red bars are indicating negative plants after four-weeks latency after the inoculation, while orange bars are indicating positive plants. The total number of plants exposed to the different combination of experimental conditions is reported above the graph (sum of the two experimental replicates).

4.3 Optimization of a ddPCR method for ToLCNDV absolute quantification

In order to measure the absolute viral load within infected samples, LIST has developed a molecular method based on the droplet digital PCR (ddPCR).

ddPCR is an advanced molecular technique for the precise and sensitive quantification of nucleic acids. Unlike traditional PCR, ddPCR partitions the sample into thousands of nanoliter-sized droplets, each acting as an independent PCR reaction. This approach allows absolute quantification by counting positive droplets containing the target DNA without the need for external standards or calibration curves. ddPCR offers unparalleled sensitivity and accuracy, making it ideal for quantifying viral loads.

LIST started the optimization from the selection of targets contained in ToLCNDV-positive samples, extracted from plant tissues, namely regions of the viral component DNA-A and DNA-B (Ferro et al., 2021; Simon et al., 2018), and a region within the elongation factor 1 alpha (EF1α) gene in the zucchini genome (Obrero et al., 2011).

These regions were selected from literature (Ferro et al., 2021; Obrero et al., 2011; Simon et al., 2018), using the reported primer pairs to amplify the specific regions. ddPCR probes targeting the selected regions were designed by LIST.

The preliminary results of the Triplex ddPCR are reported in Figure 9. The method was able to identify ToLCNDV-infected plants, showing droplets positive to ToLCNDV DNA-A and DNA-B, and the plant gene EF1α, during the same run.

In the next months LIST will focus on the final optimization of the ddPCR method, in order to publish a detailed methodological article proposing a new method for ToLCNDV absolute quantification.

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5 ESTIMATING THE EFFECT OF TEMPERATURE ON TOLCNDV

5.1 Estimating the effect of temperature on the symptomatology and accumulation of ToLCNDV

INRAE studied the effect of temperature on the accumulation and symptomatology of ToLCNDV in melon. Two isolates of ToLCNDV were tested, one with a severe (SEV) phenotype, and one with a "recovery" (REC) phenotype, characterized by decrease of symptom severity over time and observed in France since 2020. Two temperature ranges were tested, 20-25°C and 25-30°C. Symptom severity of the REC isolates was similar at both temperatures. However, the symptoms of the SEV isolate were more severe at 20-25°C than at 25-30°C. The effect of virus infection on plant growth was measured by comparing plant length and weight of infected vs. healthy plants. Plant growth was higher at high temperature, both in healthy and infected plants. The percentage of growth reduction in infected plants was not different at both temperatures. Viral accumulation was measured by qPCR on both DNAs at 21 and 35 days post inoculation (dpi) in the younger leaves of infected melons. Viral accumulation of both DNA-A and DNA-B was higher at the lower temperature 3 weeks post inoculation, whereas the difference between the different treatments was not significant at 5 weeks post infection. Thus, in the conditions tested, higher temperatures that can be expected with the climate change are not associated with more severe viral symptoms or enhanced ToLCNDV multiplication.

5.2 Estimating the effect of abiotic stresses on the durability of resistance to ToLCNDV

Melon accessions resistant to ToLCNDV (Romay et al., 2019) were mechanically inoculated at INRAE with ToLCNDV at two temperature ranges: 20-25°C and 25-30°C. Part of the plants at both temperature ranges were also submitted to drought stress by limited watering. Symptoms were observed up to five weeks post inoculation, and the presence of ToLCNDV was tested by DAS-ELISA.

Resistance breaking was not observed both during drought and temperature stress, indicating the durability of the resistance in the changing climatic conditions.

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ANNEX 1: Pictures from the experiments conducted within Task 4.3

Figure 6. ToLCNDV symptoms on zucchini plants (*Cucurbita pepo* cv. Amorgos) after agroinoculation with *Agrobacterium tumefaciens* and ToLCNDV-ES infectious clones (Jesús Navas-Castillo and Elvira Fiallo-Olivé, CSIC, Spain). These plants, reared in the two different climates and tested PCR-positive for ToLCNDV, were used as source of acquisition (for the Acquisition Access Period, AAP) for *Bemisia tabaci* used in the experiments presented above.

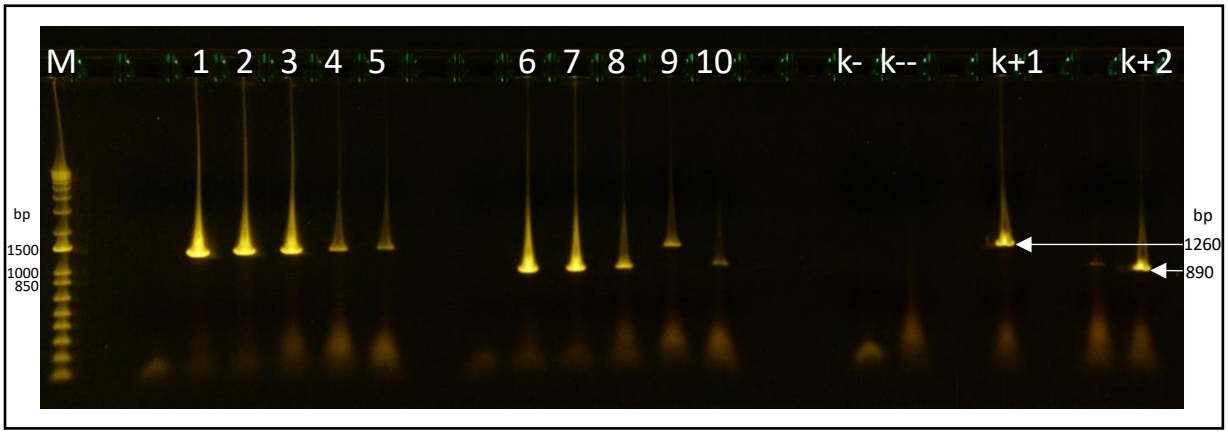

Figure 7. Endpoint PCR diagnosis of ToLCNDV infected plants, by detection of the specific fragments of ToLCNDV-DNA-A and DNA-B in symptomatic leaves of agroinfected plants 4 weeks after the inoculation. M: molecular weight marker DNA Ladder (Invitrogen™ E-Gel™ 1 Kb Plus DNA Ladder, Thermo Fisher Scientific). Lanes 1-5: DNA-A fragments (1260 bp) of five infected plant samples; lanes 6-10: DNA-B fragments (890 bp) of the same five infected plant samples (lane 9 sample: DNA-A); k-: DNA extraction negative control; k--: PCR negative control; k+1: DNA-A positive control (ToLCNDV-DNA-A transformed *A. tumefaciens*); k+2: DNA-B positive control (ToLCNDV-DNA-B transformed *A. tumefaciens*).

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Figure 8. Overview of the acquisition and inoculation experiments run in the two different climates. Briefly, after *B. tabaci* AAP, zucchini plants at the first leaf stage in both climates were exposed to five infectious *Bemisia tabaci* adults for different times depending on the experiment, constrained with clip cages under the zucchini leaf (abaxial surface). At the end of the inoculation (Inoculation Access Period, IAP), whiteflies were collected and sampled, leaving the plants for a four-weeks latency period before sampling and nucleic acids extraction, followed by PCR diagnosis.

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ANNEX 2: Preliminary results of the ddPCR optimization for ToLCNDV absolute quantification

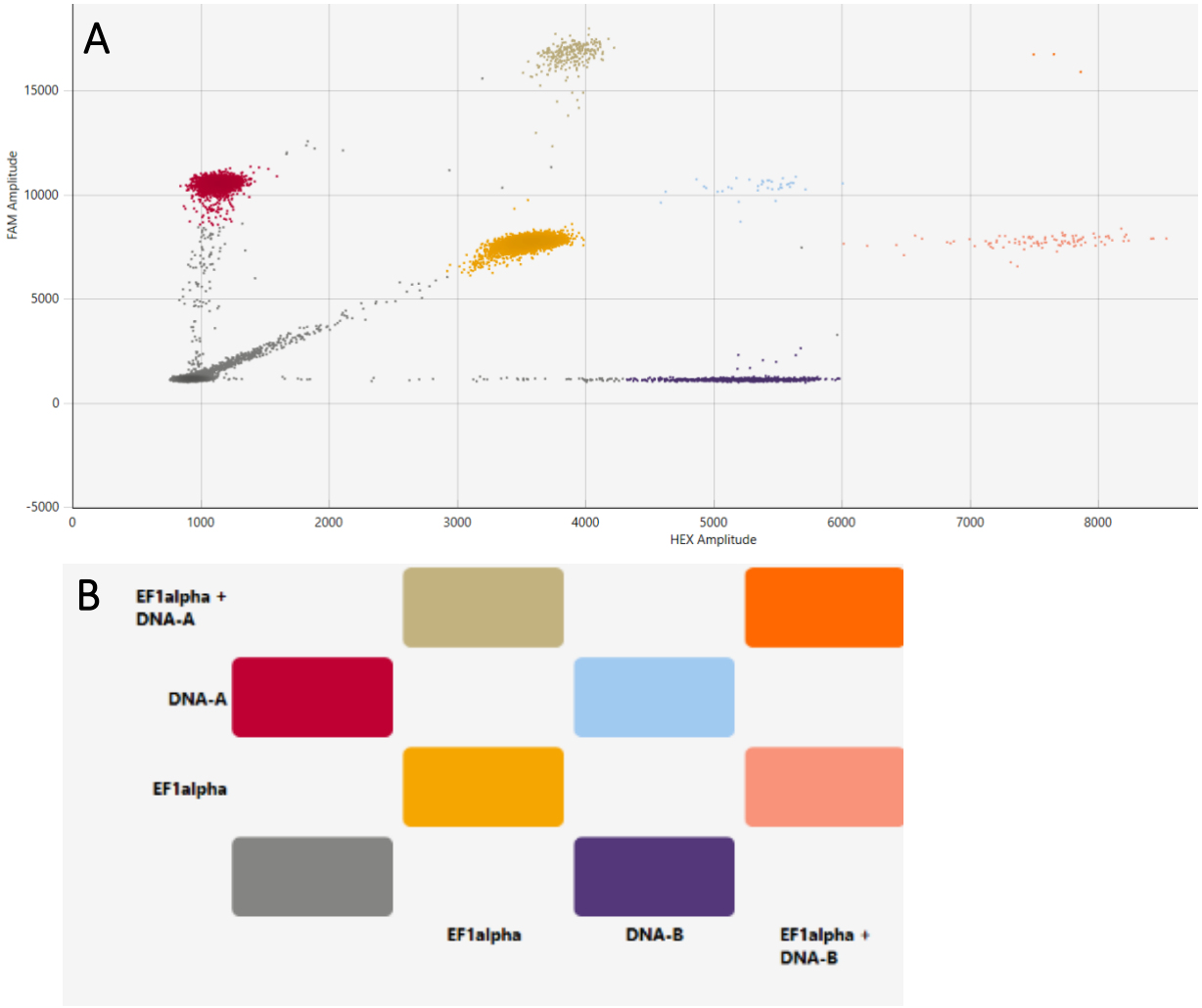

Figure 9. Triplex ddPCR (on Bio-Rad QX200) run targeting three targets: ToLCNDV DNA-A (FAM) and DNA-B (HEX), with the zucchini endogenous control gene EF1α (FAM-HEX). The graph (**A**) is presenting the bidimensional amplitude of two channels (FAM, y-axis; HEX, x-axis) in a ToLCNDV positive sample, extracted from a ToLCNDV-infected zucchini. The legend (**B**) is reporting all the different combinations that a single droplet of the run may have had: negative to all three targets (grey), DNA-A positive (red), DNA-B positive (purple), DNA-A and DNA-B positive (light blue), EF1α positive (yellow), EF1α and DNA-A positive (beige), EF1α and DNA-B positive (light orange), and positive to all three targets (orange).

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