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Proceeding Paper

Plant Disease Symptomatology: Cucumber Green Mottle Mosaic Virus (CGMMV)-Infected Cucumber Plants Exposed to Fluctuating Extreme Temperatures †

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Abstract: Greenhouse-grown cucumber plants inspected during and following extreme variations in environmental temperatures showed new characteristics of cucumber green mottle mosaic virus (CGMMV) disease manifestations. An increasing occurrence of CGMMV disease recovery has been associated with a new phenotype identified at early stages of a reemerging disease. Symptoms of bright yellow islands (BYIs), conspicuous amid a dark green surrounding tissue (DGS), were detected in up to 10% of symptomatic plants in net-houses showing 50–60% recovery following an extreme temperature wave. Importantly, similar CGMMV disease initiation stages were observed in infected cucumber plants exposed to low temperatures of ~16 °C, under conditions of both controlled growth chambers and a net-house exposed to environmental temperature fluctuations. Apparently, a wide range of fluctuating temperatures evoked gradual manifestations of a reemerging disease.

Keywords: tobamovirus; viral disease progression; disease symptom recovery; fluctuating environmental temperatures; systemic infections

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1. Introduction

Cucurbit disease caused by cucumber green mottle mosaic virus (CGMMV), first described in cucumbers in England in 1935, has recently spread globally, manifested in unmarketable fruits worldwide [1,2]. CGMMV is a seed borne virus that belongs to the genus *Tobamovirus* in the *Virgaviridae* family, which comprises highly stable mechanically transmitted viruses [3]. Disinfection studies of CGMMV-infected cucurbit seeds were unsuccessful due to virus penetration to the perisperm endosperm envelope located underneath the seed coat specifically in cucurbits [4–6]. Consequently, recent studies of CGMMV disease focused on three major directions: (1) careful hygienic conducts combined with virus inactivation tests using various chemicals [7,8]; (2) selections of tolerant or resistant genotypes that could serve as rootstocks or cultivated plants [9–13]; (3) molecular regulation studies of early disease stages that could pinpoint strategies for enhancement of plant resistance [14–18]. Importantly, all three approaches depend on precise and detailed description of early disease manifestations that could have been profoundly affected by the fluctuating extreme environmental temperatures occurring globally.

Plant molecular mechanisms affected by high temperatures were associated with different viral disease manifestations. Symptom recovery; early manifestations of dark green islands (DGIs), which indicate islands of un-infected leaf areas surrounded by systemic infections; or initiation of systemic infections in resistant plants exhibiting a hypersensitive response, were several reported contradictory manifestations that could be associated with the fluctuating surrounding conditions [19–21]. Importantly, the statistically significant higher diurnal temperature range (DTR), measured since the 1990s [22], exposes field grown crops to short-time temperature waves. We have recently shown that new early manifestations of CGMMV disease occurred in cucumbers following a recovery stage induced by an abrupt temperature raise from 25 °C to 32 °C. The new early disease stage phenotype of confined bright yellow islands (BYIs) surrounded by a dark green tissue was followed by early stages of systemic viral infections showing BYIs with diffused boundaries associated with increasing virus titer in the corresponding dark green surrounding (DGS) tissues. The newly identified phenotypes were observed under both controlled temperature conditions and in greenhouses exposed to environmental temperature changes. The early and late BYIs were confirmed as indicators of CGMMV disease initiation by biological assays. Molecular analyses of the early symptomatic cucumber plants revealed distinct characteristics for the two early stages. A consecutive up- and down-regulation of jasmonic acid (JA) signaling and increasing JA inhibitory pathways have occurred upon progression from early post-recovery stage BYIs (EBYIs) to late post-recovery stage BYIs (LBYIs). Interestingly, the cucumber plant response at the LBYI stage was associated with increasing activity of the phenylpropanoid pathway in the corresponding DGS tissues, apparently indicating high activity of plant resistance pathways towards the disease including increasing levels of reactive oxygen species as well as interference with tobamovirus accumulation [18]. We have now expanded our study to include low temperatures of ≤16 °C in monitoring the effects of high DTR on CGMMV disease symptom manifestations at a post-recovery stage. Our observations included CGMMV disease progression under both controlled fixed temperature conditions and net-house growing conditions, the latter simulated field grown cucumbers exposed to gradual and continuous changes in the DTR. Apparently, the newly identified early manifestations of CGMMV reemerging disease in cucumbers were not limited to fixed temperature manipulations but were clearly associated with the modified environmental DTR encompassing a wide temperature array.

2. Materials and Methods

Cucumber plants *cv*s. Romi and Senyal were cultivated as previously described, and inoculated with CGMMV-Rd purified inoculum [18]. Treated plants were grown in temperature-controlled growth chambers and disease symptom manifestations were monitored closely. An abrupt temperature raise effect was studied by changing the growing temperatures from 25 °C to 32 °C in 15 min. at 7 days post-inoculation before symptom development. Following viral systemic infection stage, a high temperature induced recovery stage was observed and symptomatology of the reemerging disease was recorded. For studies of low temperature effects on symptomatology of the reemerging CGMMV disease, inoculated plants showing LBYIs following the abrupt temperature raise were transferred to 16 °C-controlled growth chambers. For biological assay of EBYIs at 16 °C, EBYIs were dissected (each BYI of 3 mm in diameter) and used as the inoculum source for cucumber plants *cv*. Senyal, grown in 16 °C-controlled growth chambers. Concomitantly, the whole DGS tissue associated with the EBYIs served as inoculum source to ensure infectious potential test for the almost undetectable virus in the DGS at the early stage of the reemerging CGMMV disease.

Serological analyses were performed as previously described using ELISA tests for systemically infected plants and western blots for dissected equal sizes of EBYIs and the corresponding DGS tissues [18]. Meteorological data was extracted from records in Israeli

Meteorology Services (IMS) website: https://ims.gov.il/he/ObservationDataAPI (accessed on).

3. Results and Discussion

Disease severity of virus-infected crops have been highly affected by environmental conditions, primarily associated with modified temperatures. We have recently shown that daily extreme temperature waves that ranged between ambient temperatures of ~25 °C to above ~30 °C were accompanied by increased occurrence of disease recovery and most importantly, the reemerging disease had a profound different rhythm and new early disease manifestations were apparent [18]. Figure 1 shows a collection of symptomatic cucumber plants observed in a commercial greenhouse following daily waves of high and ambient temperatures during summer time in Israel (at Achituv village, central Israel). Systemically CGMMV infected plants were clearly apparent alongside plants showing disease recovery in apical and newly developed leaves (Figure 1a,b). Newly developed leaves in adjacent plants showed BYIs with confined boundaries containing CGMMV that was not detected in the associated DGS tissues (Figure 1c,(d1,d2)).

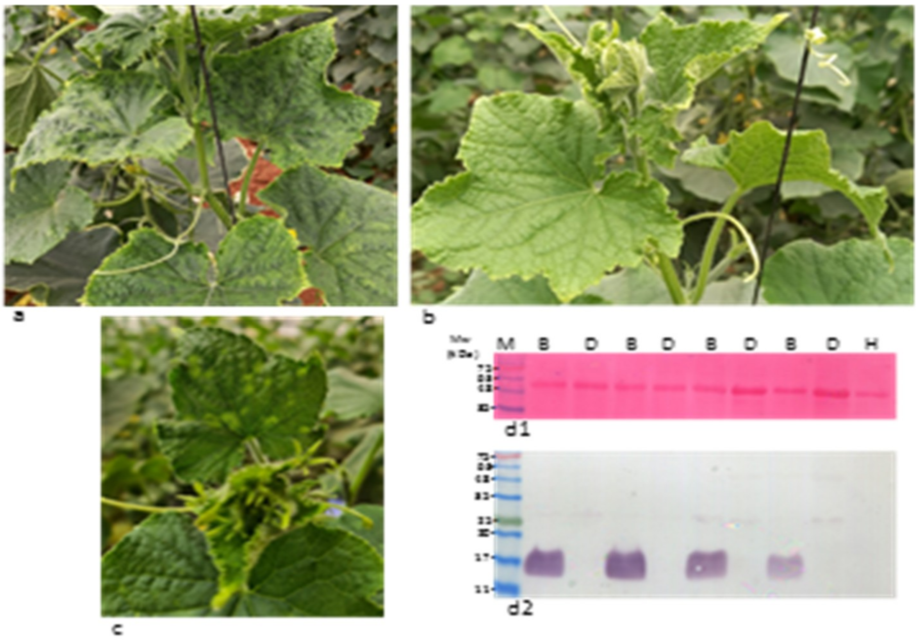

Figure 1. Field collected CGMMV infected cucumber plants following fluctuating extreme temperature events, ranging between ~20 °C and ~30 °C, during summer time in Israel. (**a**) Plants showing systemic infections. (**b**) Plants showing disease recovery in apical and young leaves. (**c**) Plants showing EBYIs. (**d1**,**d2**) Western blot showing CGMMV-CP detected only in the EBYIs. M, molecular size marker; B, EBYIs; D, DGS tissues; H, healthy controls.

In a controlled experiment that dissected the effects of high temperature fluctuations on CGMMV disease progression in cucumbers, we have confirmed that the newly identified early symptomatic stage of EBYIs was a manifestation of a reemerging disease in previously identified recovered leaves. In the experiment presented in Figure 2, CGMMV-infected cucumber plants, grown at 25 °C in temperature controlled growth chambers, were subjected to an abrupt temperature raise to 32 °C and 4–6 hs post temperature raise (hptr) recovered leaves showed EBYI manifestations.

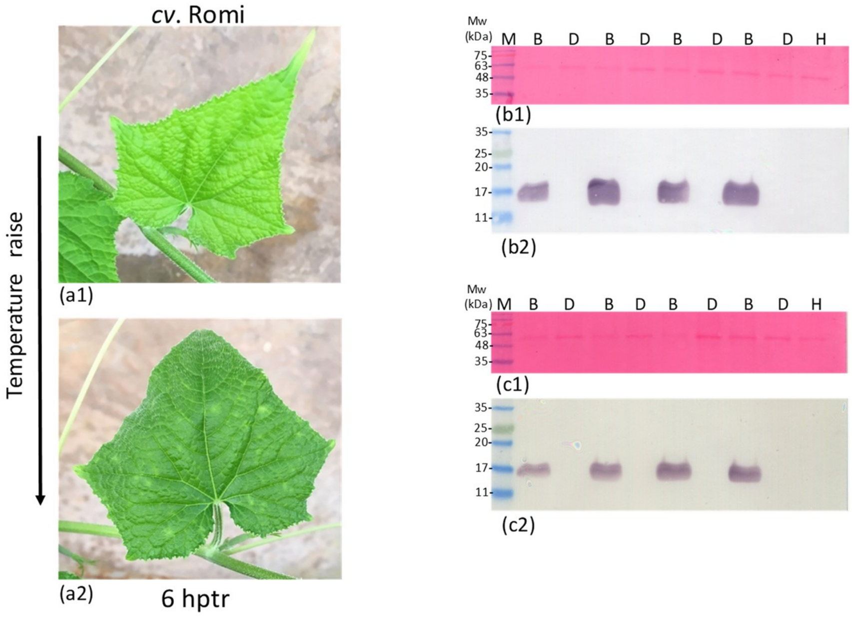

Figure 2. An abrupt temperature raise effect on post-recovery reemerging disease symptoms showing EBYI phenotypes. (**a1**,**a2**) Disease- recovered plant leaves developed EBYIs. (**b**,**c**) Western blot of an array of plants showing CGMMV-CP only in the EBYIs and not at the associated DGS tissues, which were observed at early post-recovery reemerging disease. M, molecular size marker; B, EBYIs; D, DGS tissues; H, healthy controls.

In our recent study, we have shown that EBYIs were indeed early manifestations of post-recovery CGMMV reemerging disease, which were followed by LBYIs that were associated with increasing CGMMV-titter in the corresponding DGS tissues. Importantly, those two early disease stages had distinct plant molecular profiles associated with cucumber plant response to CGMMV infections. Evidently, the precise symptomatology of viral disease progression could define the appropriate time windows for various intervention strategies [18]. Consequently, we have attempted to better define the circumstances that could promote manifestations of the early symptoms of the reemerging CGMMV disease. Importantly, increasing DTR since the 1990s that was measured in several countries in Europe was not confined to a specific climate and therefore could include a wide temperature range. Accordingly, we have tested CGMMV disease symptomatology under conditions of a variable DTR that was comprised of lower temperatures than ~25 °C. We have subjected CGMMV-infected cucumber plants that showed LBYIs, occurred in response to an abrupt temperature raise from 25 °C to 32 °C, to 16 °C growing conditions in temperature controlled growth chambers. Interestingly we have observed that the extreme temperature reduction was associated with disease recovery observed in apical and newly developing leaves (Figure 3(a2,a3)).

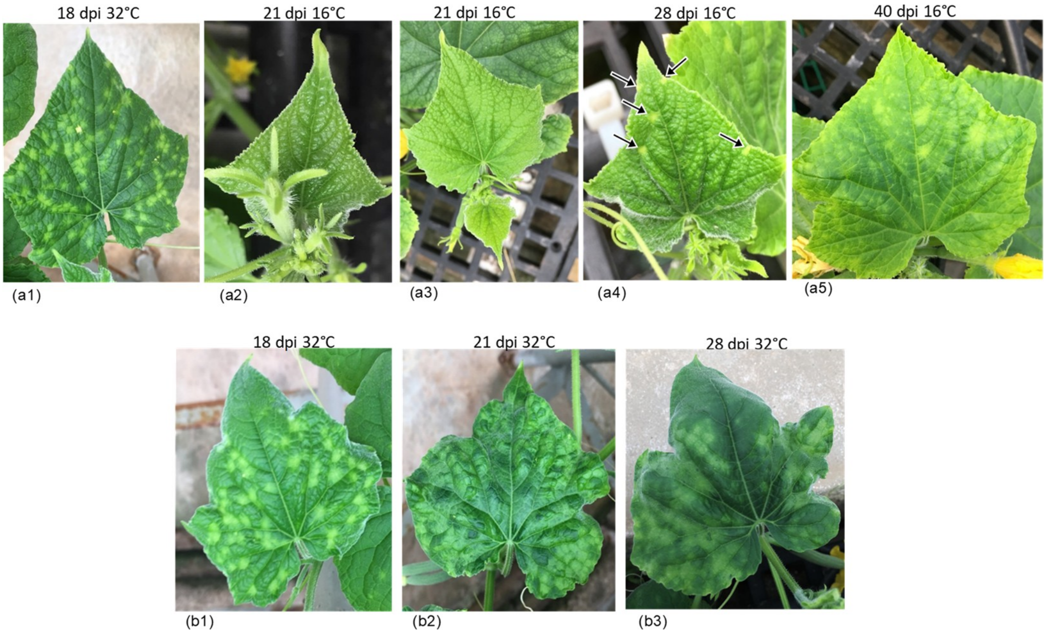

Figure 3. Low temperature effects on BYI manifestations at early stages of the CGMMV reemerging disease. (**a1**) Cucumber plants showing LBYIs at a post-recovery reemerging disease stage, following an abrupt temperature raise, transferred to 16 °C-controlled growth chambers. (**a2**,**a3**) The transferred plants, grown at 16 °C, showing CGMMV disease recovery in apical and newly developed leaves. (**a4**) EBYI manifestations at a post-recovery stage of cucumber plants grown at 16 °C. (**a5**) Severe disease mosaic symptoms of the reemerging disease at 16 °C. (**b1**–**b3**) CGMMV inoculated cucumber plants that showed LBYIs following an abrupt temperature raise, developed severe disease symptoms when kept at 32 °C. The recovery stage was followed by appearance of EBYIs in newly developed leaves (**a4**). We have confirmed that EBYIs, dissected from CGMMV-infected plants during early reemerging disease symptoms, were infectious in a biological assay of cucumber plants grown at 16 °C-controlled growth chamber. EBYIs initiated systemic viral infection although plant growth and disease progression were attenuated. In order to determine the applicability of the observed results we have monitored symptomatology of CGMMV disease progression in virus-infected cucumber plants grown in a net house that simulated field conditions of plant-exposure to environmental variable temperatures. We have observed that although systemic viral infections have occurred at 14 days post inoculation (dpi), fluctuating DTR that included high temperatures of ≥30 °C were not necessarily associated with manifestations of a recovery stage and associated new early BYI symptoms of a reemerging disease until 29 dpi. Importantly, an abrupt reduction of daily low temperatures to 17 °C and 15 °C, at 30–31 dpi, with no associated increase in DTRs, was followed by early appearance of disease recovery in apical and newly developed leaves (Figure 4). At 33 dpi, CGMMV disease recovery was observed in apical leaves adjacent to plants showing EBYIs. Importantly, at 34–35 dpi, 60 out of 77 plants showed disease recovery in apical leaves and ~50 plants showed BYIs in at least one leaf.

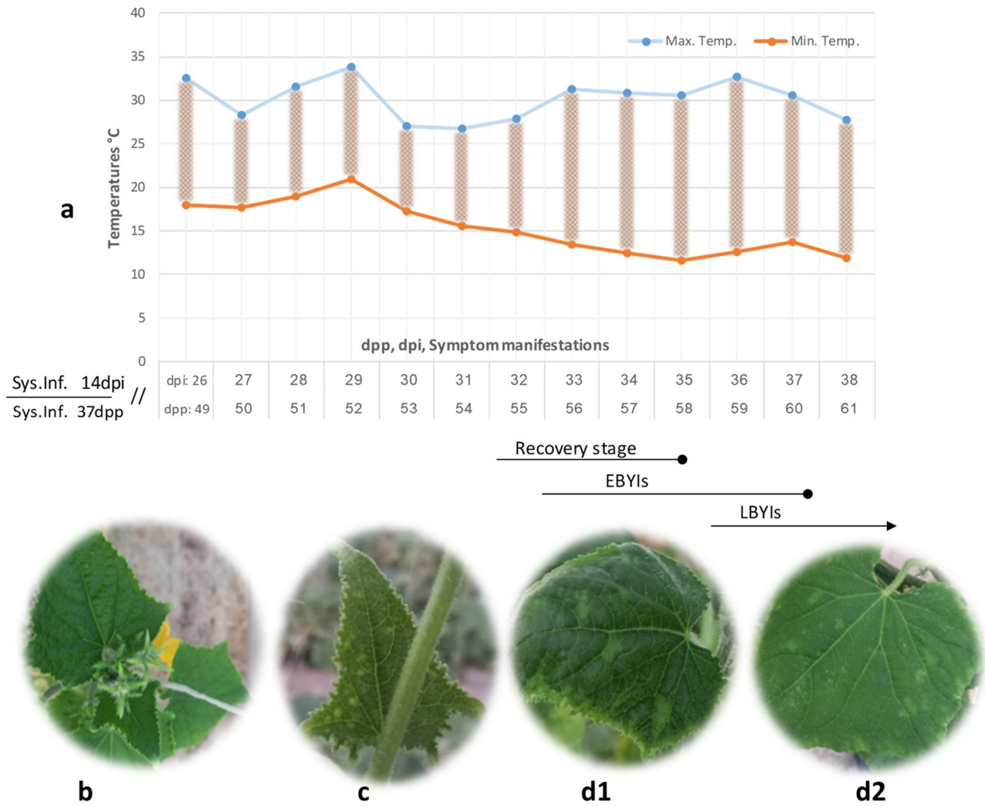

Figure 4. CGMMV disease symptomatology in cucumber plants grown in a net-house, simulating field-grown crops exposed to environmental temperature fluctuations during autumn, in Israel. (**a**) A graph showing records of fluctuating DTR following CGMMV systemic infection that was observed at 14 dpi. (**b**) Plants showing disease recovery in apical and newly developed leaves. (**c**) Manifestations of EBYIs in apical and newly developed leaves. (**d1**,**d2**) Manifestations of LBYIs.

4. Conclusions

Our data suggest that extreme changes in temperatures, either high or low, could contribute to a new reemerging disease pace manifested in BYI symptoms following a recovery stage. A wide array of environmental temperatures could play a role in the increasing DTR effects on plant susceptibility and/or symptom manifestations.

Conflicts of Interest: The authors declare no conflict of interest.

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