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Ambient conditions of elevated temperature and CO_2 levels are detrimental to the probabilities of transmission by insects of a *Potato virus Y* isolate and to its simulated prevalence in the environment

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ABSTRACT

Conditions of elevated temperature and CO_2 levels [30 °C and 970 parts-per-million (ppm), respectively] reduced the systemic titers of a potato virus Y (PVY) isolate in *Nicotiana benthamiana* plants, relative to standard conditions (25 °C, ~405 ppm CO_2). Under controlled conditions we studied how these growing environments affected the transmission of infection by aphids. Probabilities of transmission of infection by insects that fed on infected donor plants kept at either standard conditions, or at 30 °C and 970 ppm CO_2 were both determined and found to positively correlate with titers in donor leaves, independently of the ambient conditions in which recipient plantlets would grow. With these data, viral prevalence was simulated under conditions of elevated temperature and CO_2 levels and found that for it to remain comparable to that simulated under standard conditions, insect arrivals to recipient plants in the former scenario would have to increase several-fold in their frequency.

1. Introduction

Abiotic parameters influence outcomes of infection of plants by viruses in ways that are specific to each particular interaction. For example, ambient conditions of relative elevated temperatures have been known to attenuate systemic symptoms in many compatible infections by positive-sense RNA viruses, and were usually associated to decreases in viral titers (De Bokx and Piron, 1977; Hull, 2002; Szittya et al., 2003; del Toro et al., 2015, 2018; Aguilar et al., 2015; Chung et al., 2016). However, in other infections elevated temperature had either little or no detrimental effect on viral titers and on disease symptoms, or even potentiated them, as was the case in the infection of *Nicotiana benthamiana* plants by the cucumber mosaic virus (CMV) strain Fny (del Toro et al., 2015). Different strains from the same virus species could react to elevated temperatures in completely different ways, with regard to symptoms and/or viral titers, as was shown for different strains of CMV that infected muskmelon plants (Roossinck, 1991), or for different strains of the *Potyvirus* potato virus Y (PVY) infecting *N. benthamiana* plants (del Toro et al., 2015; Chung et al., 2016), indicating that temperature effects are not determined at the virus species taxon.

Both elevated temperature and CO_2 levels are ambient abiotic parameters associated to climate change. Ambient CO_2 levels are expected to increase steadily during the rest of the century under any of the anthropogenic emissions scenario contemplated, and radiative forcing by this and other greenhouse gasses is very likely going to increase global average temperatures, and the frequency and extent of heat waves (IPCC, 2014 synthesis report. Pachauri and Meyer Eds. http://www.ipcc.ch/;). Several works have studied how elevated temperature and/or higher levels of CO_2, as well as other abiotic parameters affect compatible infections of plants by different RNA viruses, with regard to viral titers and the symptoms induced (Matros et al., 2006; Ye et al., 2010; del Toro et al., 2015, 2017; Zhang et al., 2015; Chung et al., 2016; Dáder et al., 2016; van Munster et al., 2017). Results from those works suggest that climate change will affect plant RNA virus isolates and strains differently in their abilities not only to infect and potentially cause damage to crops, but also to establish infection, disperse and ultimately remain in the environment.

Most plant viruses disperse by insect vectors (Brault et al., 2010; Froissart et al., 2010). Extensive literature exists on studies concerning the transmission of plant viruses by insect vectors. Those studies show

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that transmission probabilities for a certain virus species can be affected by the virus strain and the specific compatible host plant it infects, and also by the biotype of the insect vector species that transmits it (for reviews, see Fereres and Moreno, 2009; Froissart et al., 2010; Trębicki et al., 2016; Whitfield et al., 2018).

Many RNA viruses are transmitted by aphids in a non-persistent manner, including potyviruses and cucumoviruses (Watson and Roberts, 1939), which constitute two of the most important genera of plant RNA viruses because of their economic impact, cosmopolitanism in number of plant species that they can infect, and global geographical distribution (Hull, 2002). Numerous studies on the non-persistent transmission of RNA viruses have been published. Some of those studies have compared relative transmission frequencies of different virus species by the same aphid biotype (Chung et al., 2016). Other studies have compared probabilities of transmission by the same aphid biotype of different mutant variants of the same virus species (Atreya et al., 1991, 1992; Huet et al., 1994; Peng et al., 1998; Llave et al., 2002), of different natural strains of the same virus species (Normand and Pirone, 1968; Baulcombe et al., 1993; Canto et al., 1995; Llave et al., 1999), or of virus isolates that had or had not associated components that influenced viral titers, such as satellite RNAs (Escriu et al., 2000). Only a few studies have determined probabilities of transmission by the same insect vector of a virus isolate that had significantly different viral titers in the same host plant, for whatever the reason (De Bokx et al., 1978; Banik and Zitter, 1990). Additional studies on the effect on transmission of differences in viral titers were also been made using artificial membrane feeding experiments and purified virion preparations (Pirone and Megahed, 1966; Gera et al., 1979). Overall, in the majority of the above-mentioned works lower virus titers resulted in reduced transmission efficiencies, although correlations between titers and transmission efficiencies were not always established and some discrepancies were also observed. The mechanistic reason behind a link between lower viral titers and lower transmission is not obvious, since the number of virions involved in non-persistent aphid transmission events appears to be very low. In the case of PVY, that number was estimated at 0.5–3.2 particles/insect and transmission event in pepper plants (Moury et al., 2007), which is extremely low when compared to the number of particles that would be present in the intracellular sap being probed by the insect (Martín et al., 1997; Powell, 2005). Binding of virions plus the HCPro co-factor to putative receptors in the aphid stylet might not become saturated in conditions of low viral titers, despite an expected excess in molar amounts of viral particles in the sap relative to receptor sites in the insect. Thus, although the process of non-persistent viral transmission by insects is a likely product of virus-insect-host co-evolution, and is based on specific molecular interactions (Atreya et al., 1990, 1991; Ammar et al., 1994; Blanc et al., 1998; Peng et al., 1998; López-Moya et al., 1999; Uzest et al., 2007, 2010; Fernández-Calvino et al., 2010), it may not be very efficient.

In previous works we had found that an isolate of PVY decreased its systemic titers in the compatible host *N. benthamiana* when plants were kept under what we called climate change-associated (CC) conditions of elevated temperature (30 °C) and CO_2 levels [970 parts-per-million (ppm)], relative to those found in plants kept under standard conditions of 25 °C and ~405 ppm ambient CO_2. This drop in viral titers was permanent and lasted for the life-span of the plant (del Toro et al., 2018). Here we have experimentally measured under controlled conditions how these two altered abiotic parameters affected the probabilities of viral transmission by aphids from infected plants kept under those conditions, and how in our experimental patho-system titers and transmission probabilities related to each other. With those data we simulated the effect of titers under each ambient condition on infection dispersal and its prevalence in the environment through time.

2. Materials and methods

2.1. Plants and viruses

N. benthamiana plants were used in this study. Plants were seeded and germinated under st conditions. After they were individually potted they were transferred for growth to controlled growth chambers with a photoperiod of 16/8 h (day/night) and a daylight intensity of ~2500 luxes. Plants were kept in either of two environment conditions, with regard to temperature and CO_2 levels: standard (st) conditions of 25 °C and current atmospheric CO_2 partial pressures (~405 ppm), and conditions of elevated temperature (30 °C) and CO_2 partial pressures of ~970 ppm [climate change-associated (CC) conditions]. To achieve these two conditions and made comparison as accurately as possible we used two identical phytotrons (Environmental Test Chambers model MLR-350, Sanyo Corp. Japan) that were both fitted by our Institute´s Technical Service with prototype devices that accurately regulate ambient CO_2 levels in the chambers, using an internal CO_2 sensor (Calitrans 300, Leyro Instruments, Spain) and a continuous CO_2 dispenser (model Leonardo. Arduino, Italy) from external CO_2 canisters. Lighting was the same in both machines and temperatures were set accordingly for either st or CC conditions.

An aphid-transmissible Scottish ordinary variety (O) isolate from *Potato virus Y* (PVY) was used (Barker et al., 2009; genbank accession number of the full-length sequence AJ585196). A single extract from infected plant tissue was made in phosphate buffered saline, pH 6.8, at 10% (w/v), aliquoted and kept at −80 °C, and used as inoculum source for all mechanical inoculations. 2-to-3-week old plantlets were dusted with carborundum (Carlo Erba, Barcelona, Spain) and rubbed with 30 µl of inoculum. In those experiments in which plants were to be grown under CC conditions, plants were pre-acclimated to those conditions for one week before inoculation with virus extract.

2.2. Aphids and aphid transmission assays

For aphid transmission assays a clonal population of females of the peach aphid *Myzus persicae* (Sulzer) (*Insecta: Hemiptera: Aphididae*) was used. This population has been indefinitely maintained by parthenogenesis on tobacco plants (*Nicotiana tabacum*) in an insectary under st conditions.

All transmission assays were performed in the laboratory under st conditions. Systemically-infected leaves used as donors were fully expanded upper leaves obtained from plants that had been inoculated 14 days before with virus extract and had been kept growing at either st or CC conditions. Donor leaves were detached and washed gently with a mild aqueous solution of 0.5% Tween® 20 (Sigma Aldrich, St. Louis, USA) to remove any surface irritants to aphids. Leaves were placed inside a glass container over wet filter paper with their abaxial side upwards. Second and third instar aphid nymphs from the insectary colony were collected with the help of a paint brush, deposited inside small glass bottles, and left starving for 3 h. Aphids were then placed on the donor leaves where they were allowed to feed for 15 min. With the help of a paint brush aphids were then individually transferred to small healthy *N. benthamiana* receptor plantlets. The number of aphids that each plantlet received varied with each experiment as is also indicated in the corresponding Figure legend: In the experiments shown in Fig. 1, two independent replica experiments were performed, with 40 plants used per replica, 10 plants were used for each of the recipient number of aphids assessed (1, 2, 3 or 4 aphids/recipient plantlet). In total 80 plants and 200 aphids were used; In the experiment shown in Fig. 2 from each of the eight donor leaves aphids were transferred to 10 plantlets (4 aphids/recipient plantlet). In total, 80 plantlets and $4 \times 80 = 320$ aphids were used in the experiment; In the experiment shown in Fig. 3 from each of the 16 donor leaves aphids were transferred to 10 plantlets (3 aphids/plantlet). In total 160 plantlets and $3 \times 160 = 480$ aphids were used. Every plantlet was individually

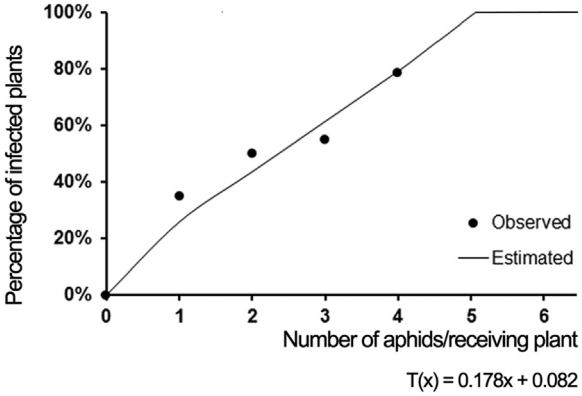


Fig. 1. Frequencies of transmission of a potato virus Y (PVY) isolate by peach aphids (*Myzus persicae*) that fed on donor leaves from infected *Nicotiana benthamiana* plants that had been kept under standard (st) conditions of 25 °C and current atmospheric levels of CO_2 (~405 parts-per-million), using different numbers of aphids/recipient plantlets. Viral titers in donor leaves estimated by RT-qPCR were 1.02 (standard deviation of 0.07) relative to the external control (a pool of total RNAs from several infected leaves grown under standard conditions, and the average level of PVY genomic RNA obtained by RT-qPCR from that sample was given the arbitrary value of 1). Transmission frequencies showed strong positive linear correlation with the number of aphids/recipient plantlet (Pearson´s r = 0.965, P-value < 0.01). Dots represent averaged values empirically obtained in two independent replica experiments, 40 plants were used per replica, 10 plants/each of the receiving number of aphids assessed (1, 2, 3 or 4 aphids/recipient plantlet). In total 80 plants and 200 aphids were used. This proportion is shown as percentage of infection in the chart.

covered with an inverted glass beaker to prevent aphids from moving between plants. After the transfer of insects, receptor plantlets were placed immediately in controlled growth chambers at either st or CC conditions. Aphids were allowed to feed overnight on those plantlets before being killed with insecticide the next day, as described (del Toro et al., 2014). Detection of systemic PVY infection in receptor plants grown under st conditions was performed visually after strong infection symptoms become apparent at 9 days after the transmission assay. As infection symptoms in systemically-infected plants grown under CC conditions were very mild (del Toro et al., 2018), plantlets kept under CC conditions were placed under st conditions 1 week after the transmission experiment, to allow for the development of strong visual symptoms of infection 5–7 days afterwards.

2.3. Quantification of viral titers in leaves used as donors in transmission assays, and detection of systemic infection

Relative viral titers in each of the donor leaves used in the transmission assays performed here were determined by one-step reverse transcription plus real time quantitative polymerase chain reaction (RT-qPCR) as described in del Toro et al. (2015). Total RNA was extracted from three pooled discs of 1 cm diameter each of donor leaf tissue using TRIzol reagent (Invitrogen, Carlsbad, USA) following manufacturer instructions and contaminant DNA was removed by treatment with TURBO DNA-free kit (Ambion, Austin, USA) as described (del Toro et al., 2014). RT-qPCR was performed using a final reaction volume of 15 µl that contained 7.5 µl of Brilliant III Ultra-Fast RT-qPCR Master Mix (Agilent, Santa Clara, USA), 1.8 µl of RNase-free water, 0.75 µl of reverse transcriptase (Agilent), 0.15 µl of 100 mM dithiothreitol (Agilent), 0.3 µM each primer, and 3 µl of total RNA extract (approximately 15 ng RNA/µl). Relative quantification of PCR products was calculated by the ΔΔCt method. The primers employed were PVY-Fw (5´-CTGTG GGGACAAAGGGAGTA-3´) and PVY-Rv (5´-GGATGCTTGCGGATTTC ATA-3´) for PVY, and 18SrRNA-Fw (5´-GCCCGTTGCTGCGATGATTC-3´) and 18SrRNA-Rv (5´-GCTGCCTTCCTTGGATGTGG-3´) for 18S rRNA for normalization. RT-qPCR assays were performed in a Rotor-Gene Q thermal cycler (Qiagen. Venlo, Netherlands) using the following thermal protocol: 50 °C for 10 min; 95 °C for 3 min; 40 cycles of 95 °C for 10 s and 60 °C for 20 s; and a final ramp for melting analysis from 60 °C to 95 °C rising 1 °C every 5 s. For the determination of virus titers by RT-qPCR in Figs. 1 and 3, an external control sample was included that consisted of a pool of total RNAs from several infected leaves grown under standard conditions, and the average level of PVY genomic RNA obtained by RT-qPCR from that sample was given the arbitrary value of 1, and to which titers shown in the charts refer.

To determine the length of time that it took for a plant to become systemically infected under st or CC conditions (Fig. 5A), plants were inoculated mechanically on a single carborundum-dusted lower leaf (fifth expanded leaf starting from the top) with 20 µl of PVY extract. From each inoculated plant 1 cm discs of leaf tissue were collected daily from upper expanding leaves. Leaf discs were extracted in 100 µl of extraction buffer and viral presence was assessed by 15% SDS-PAGE plus western blot as described (del Toro et al., 2018) using an antibody against the viral coat protein (CP; Llave et al., 1999). The first leaf disc that gave a positive detection of CP presence was considered as the first day in which infection became systemic.

2.4. Determination of aphid transmission probabilities under st or CC conditions, and simulation of their effect on the prevalence of infection through time

Under our experimental conditions infection by PVY of recipient plantlets through transmission by aphids showed a linear relationship with the number of aphids involved in each transmission event when donor leaves originated from plants kept under st conditions and recipient plantlets were grown under st conditions, as shown in Fig. 1. To estimate transmission probabilities, a model adjusted to a linear regression was deduced as $T_e(x) = (a \bullet x + b) \bullet R_e$, where $T_e(x)$ is the probability of PVY transmission by a variable number of aphids (x) within a range of ($0 \leq T_e(x) \leq 1$), R_e is a ratio of the relatively reduced capacity at an environment condition relative to st condition (R_e value is 1 in st condition), and a and b are parameters of the linear equation in st conditions.

A simulation on the dispersal of infection by aphids and on viral prevalence under st or CC ambient conditions (Fig. 5B) was performed using the Susceptible-Infected (SI) spread model, applying a demographic turnover (Keeling and Rohani, 2007). Briefly, assuming an initial population of N individuals of *N. benthamiana* plants was homogenously distributed into eight age ranges, from one to eight weeks old. In order to maintain a stable number of plants after the eighth week, N/8 plants were removed (death) and substituted by a new set of N/8 plantlets (from seed) that were healthy because in our model we assumed PVY infection is not transmitted vertically. Simulations were performed for a period of time of 150 weeks. In the simulation infected plants were assumed to distribute homogeneously throughout the different plant age ranges. Initial percentages of infected plants could be modified, but for the chart shown in Fig. 5 that percentage was situated at 50%. For a given number of aphids (x) reaching each plant, the number of infected plants ($I(x)$) in a week (t) was calculated as the number of plants that were infected $I_{t\text{-}1}$ when they were a week younger, plus the number of plants that were healthy when they were a week younger $H(x)_{t\text{-}1}$ multiplied by the effective transmission probabilities of those aphids ($T_e(x)$) and by the global percentage of infected plants (GI) in the previous week ($t\text{-}1$) divided by one hundred: $I(x)_t = I(x)_{t\text{-}1} + H(x)_{t\text{-}1} \cdot T_e(x) \cdot GI(x)_{t\text{-}1}/100$, formula described also in Fig. 5B.

An interactive algorithm that allows the introduction of input parameters different to those used to create the charts in Fig. 5B (such as the initial percentages of infected plants) to determine their effect in the simulation is provided as Supplemental material (Supplementary Excel. File S1).

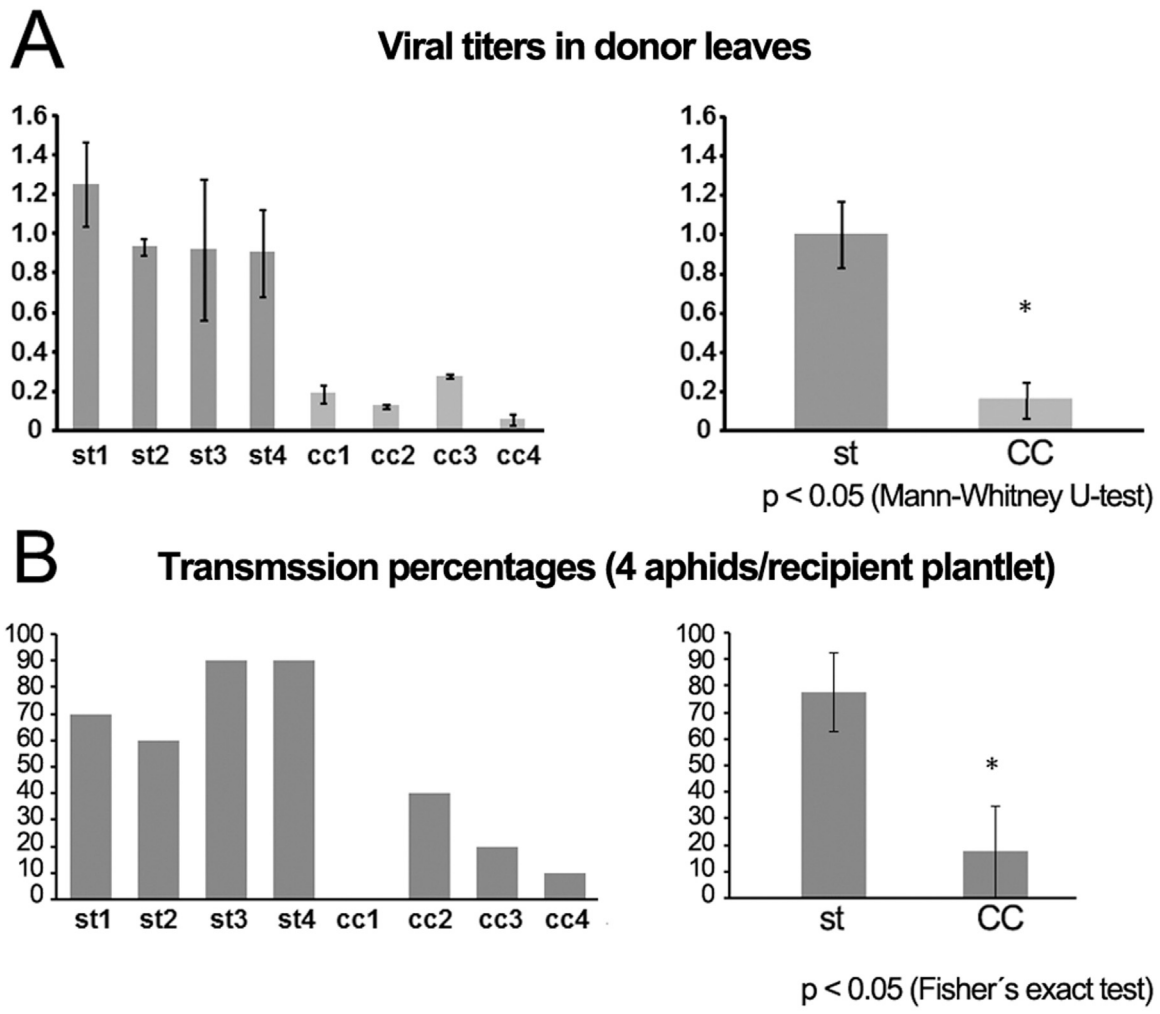


Fig. 2. The transmission of a potato virus Y (PVY) isolate by the peach aphid *Myzus persicae* when insects fed on infected donor leaves of *Nicotiana benthamiana* plants that had been kept either under standard (st) conditions of 25 °C and current atmospheric levels of CO_2 of ~405 parts-per-million (ppm), or under conditions of elevated temperature and CO_2 levels [climate change-associated conditions (CC)] of 30 °C and 970 ppm of CO_2. **A.**, determination by RT-qPCR of viral titers in the eight individual donor leaves (st1 to st4, and cc1 to cc4) when aphid transmission experiments were performed relative to the combined average of titers in the individual st leaves (average value of 1). Dark and light gray bars in the left chart correspond to titers from each individual donor leaves originating from st- or CC-kept plants, respectively. The right chart shows average viral titers for leaves from either condition. Differences in titers in leaves from plants kept under either ambient condition were significant (Mann-Whitney *U*-test, $P < 0.05$). **B.**, determination of the frequencies of transmission of PVY infection by aphids that fed on the eight, st-kept or CC-kept donor leaves whose titers are indicated in A. From each donor leaf aphids were transferred to 10 plantlets (4 aphids/recipient plantlet). In total 80 plantlets and $4 \times 80 = 320$ aphids were used in the experiment. The whole experiment was performed in the same day to minimize differences in aphid behaviour. The left chart shows the percentage of plants that became infected from each individual donor leaf. The right chart shows average percentages of infected plants.

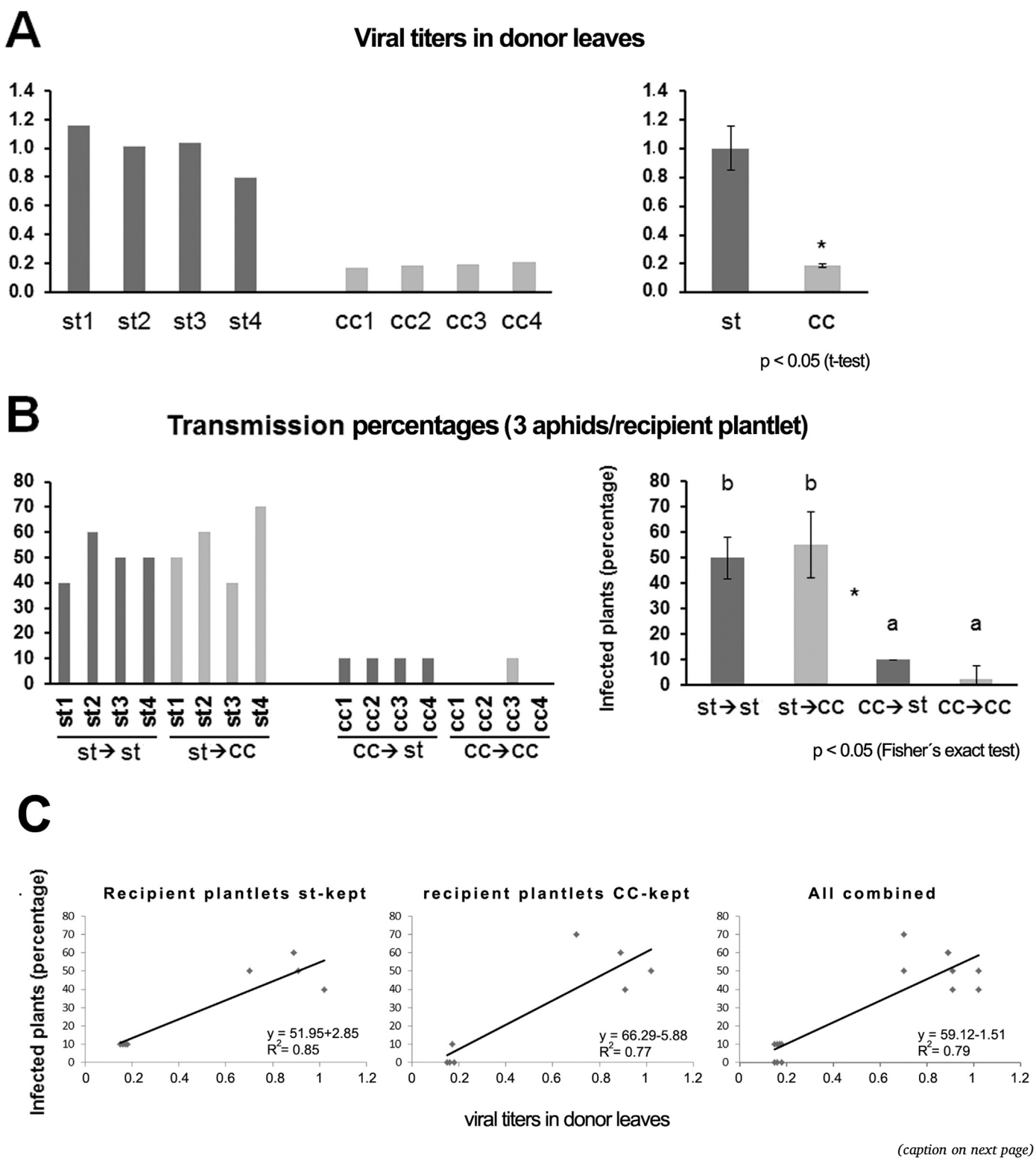
2.5. Statistical analysis

All statistical analyses in this work were carried out with SPSS Statistics (IBM, Armonk, NY, USA). Transmission efficiencies were analyzed using Fisher's exact tests at an alpha (α) level of 0.05 when two groups were compared. Bonferroni α level correction was applied (corrected α = 0.05/number of compared pairs) when more than two groups were compared. Tests applied to analyze viral titers in the donor leaves used in transmission assays were either Student's *t*-tests, or Mann–Whitney *U* tests, depending on the normality of data. A Spearman´s correlation test was used to test whether there was correlation between the number of aphids used in transmission events and the number of plants that became infected. Analysis of the time that it took for virus infections to become systemic from the initial point of inoculation under different environment conditions (Fig. 5A) were performed using Mann-Whitney *U*-tests and with students T-test with Welch correction, depending on whether data followed Gaussian distribution or not.

3. Results

3.1. Observed frequencies of transmission of infection relative to the number of aphids used, and determination of the probability that an individual aphid transmits it under st conditions

We determined the relationship between the number of potentially viruliferous aphids that landed on healthy recipient plants after feeding on a PVY-infected donor leaf from a plant maintained under st conditions and the number of recipient plants that became infected. To do that, a transmission assay with two experimental replicates was performed (Fig. 1): aphids were allowed to feed on a single PVY-infected donor leaf and were then transferred to healthy recipient plants, depositing from 1 to 4 aphids on each plant. Recipient plants were kept afterwards under st conditions. The proportion of them that became infected increased with the number of aphids that they received (Fig. 1). Transmission frequencies (defined as the percentage of plants that became infected after receiving aphids that have fed previously on



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infected plants) showed strong positive linear correlation with the number of aphids used (Pearson´s r = 0.965, P-value < 0.01).

3.2. Observed transmission frequencies when infected donor plants had been kept under either st or CC conditions

In our experimental pathosystem, we studied how aphid transmission frequencies compared when infected donor plants had been grown under CC conditions, relative to st conditions. To test this we compared transmission frequencies when infected donor leaves originated from plants grown under either st or CC conditions. We also quantified relative viral titers in each of the individual donor leaves used by RT-qPCR, and they were significantly lower in CC leaves than in st leaves (~80% lower; $p < 0.05$, Mann-Whitney *U*-test; Fig. 2A, shown in the charts as bars in light and dark shades of gray, respectively). Transmission experiments from each of those infected leaves were performed using four aphids per recipient plantlet, and showed that transmission frequencies were significantly lower when aphids fed on CC donor

Fig. 3. The transmission of a potato virus Y (PVY) isolate by the peach aphid *Myzus persicae* when insects fed on infected donor leaves of *Nicotiana benthamiana* plants that had been kept either under standard (st) conditions of 25 °C and current atmospheric levels of CO_2 of ~405 parts-per-million (ppm), or under conditions of elevated temperature and CO_2 levels [climate change-associated conditions (CC)] of 30 °C and 970 ppm of CO_2. **A.**, determination by RT-qPCR of viral titers in the eight individual donor leaves (st1 to st4, and cc1 to cc4) when aphid transmission experiments were performed (14 days post-inoculation) relative to the external control (value of 1). Dark and light gray bars in the left chart correspond to titers from each individual donor leaves originating from st- or CC-kept plants, respectively. The right chart shows average viral titers for leaves from either condition. Differences in viral titers in leaves from plants kept under either ambient condition were highly significant (Student's *t*-test, $P < 0.05$). **B.**, determination of the frequencies of transmission of PVY infection by aphids that fed either on st-kept or CC-kept donor leaves shown in A, and of the effect of growing the recipient plants after the experiment under either st or CC conditions. From each of the donor leaves aphids were transferred to 10 plantlets (3 aphids/plantlet). In total 160 plantlets and $3 \times 160 = 480$ aphids were used in the experiment. The whole experiment was performed in the same day with the same aphid batch to minimize differences in aphid behaviour. The left chart shows the percentage of infected plants that would grow under st conditions (dark gray) or under CC conditions (light gray). The right chart shows the average percentage of infected plants using recipient plantlets under st (dark gray) or cc conditions (light gray) and their standard deviation bars. Transmission percentages were significantly lower when donor leaves had been from CC-kept than st-kept plants. The environment condition in which recipient plants would grow after the experiment had no significant effect on transmission of infection. Different characters in chart indicate significant differences (Fisher´s exact test, $P < 0.05$). **C.,** transmission frequencies in recipient plants showed positive linear correlation with the titers of virus measured in donor leaves under each recipient ambient scenario, or for both combined ($P < 0.05$ in the three cases. Spearman´s correlation test).

leaves than on st donor leaves (~80% lower; Fisher´s exact test, $P < 0.05$; Fig. 2B). Although observed drops in transmission percentages and in viral titers were similar a linear correlation between transmission frequencies and virus titers in donor leaves could not be established in this particular experiment.

3.3. Determination of the probabilities of transmission of infection by aphids from donor to recipient plants kept under either st or CC conditions

In a replica experiment, we tested additionally whether the ambient conditions in which recipient plantlets would be kept after the transmission experiment (st or CC conditions) would affect transmission frequencies. As before, we used donor leaves that originated from plants grown under st as well as under CC conditions (Fig. 3; st- or CC-kept donor leaves or recipient plantlets are indicated in the charts with dark or light shades of gray, respectively). We measured viral titers in each of the donor leaves used, and found that they were significantly lower in the four donor leaves from plants kept under CC conditions than in those originating from plants kept under st conditions (Fig. 3A, dark vs. light bars, respectively). We also found that transmission frequencies decreased significantly when donor leaves originated from CC-kept plants, relative to those observed from st-kept plants. However, there were no significant differences in transmission frequencies when recipient plants were allowed to grow under either st or CC conditions after the transmission events (Fig. 3B). The frequency of transmission from st donor leaves was 52.5% (42 infected plants over a total of 80) using 3 aphids/recipient plantlet, which is similar to the one obtained in the experiment shown in Fig. 1 (55%), while that from CC donor leaves was 6.3%. Transmission probabilities from CC donor leaves were thus 0.1132 times (R_e) those measured under st conditions (0.53/0.06). Transmission frequencies showed linear correlation with the titers of virus measured in donor leaves regardless of the conditions in which recipient plantlets would grow (Fig. 3C).

3.4. The effect on the simulated prevalence of infection of the differences in transmission from donor plants grown under st or CC conditions

With the data from Figs. 1 and 3 we could calculate transmission probabilities from either st or CC donor leaves for different numbers of aphids probing on recipient plantlets (Fig. 4A). Those probabilities of transmission of infection adjust to a model $T_e(x) = (a \bullet x + b) \bullet R_e$, where the value of R_e is in st conditions 1 and in CC conditions 0.1132. Estimated values of the parameters a and b were 0.178 and 0.082, respectively ($df = 1, 3$, $F = 41.26$, $P = 0.0077$, $r^2 = 0.93$). The value of $T_e(x)$ is 0 when the number of aphids is 0 and it increases up to 1 as the number of aphid increase. Under st conditions it reaches 1 when the number of aphids is 5 or over, while it continues to increase in CC conditions (Fig. 4A). From this formula it was also deduced that under st conditions that probability for a single aphid to transmit infection after feeding on an infected plant is 0.260 (26%), while under CC conditions that probability was only 0.029 (2.9%).

The estimated number of transmission events required to infect a recipient plantlet were very different for each environmental condition, st and CC. While four events of one aphid reaching and probing a recipient plantlet after having fed on an infected donor leaf kept under st conditions would suffice to infect it (assigned value 1), if aphids fed on infected plants that had been kept under CC conditions the number of events would have to increase to 34 (Fig. 4B)

To simulate the dynamics of prevalence of infection through time under both ambient scenarios using our results on transmission probabilities we first determined experimentally the lengths of time that it took for an infection to become systemic from the initial point of mechanical inoculation in plants of the same age. We found that they did not differ significantly under st or CC conditions (Fig. 5A). We thus assumed in our model that the length of time that it took for a plant to become systemically infected after successful inoculation by viruliferous aphids was the same under either condition, throughout any plant age. We assumed that there was no spatial restriction to the visit of aphids between plants, and that the behaviour and capability to transmit the virus was similar in aphids that lived under either st or CC conditions. We also assumed that the probability of virus transmission by an aphid that had been feeding from a PVY-infected donor leaf of a plant kept under st conditions was 0.260; and that the same probability from a plant kept under CC conditions was 0.1132 times lower. The probabilities of virus transmission when more than one aphid reached a recipient plant were calculated according to Fig. 4A.

We then simulated the probability that a *Nicotiana benthamiana* plant in a population of 100 healthy plants would become infected over its lifetime of around eight weeks (55 days) when it received a variable number of aphid visits (1−40)/day, which had previously fed on an outside infected source under st or CC conditions (upper and lower charts, respectively), assuming that each arriving aphid would probe the recipient plantlet once (Fig. 5B).

Our simulation showed that under st conditions PVY disappeared from the environment when there was on average only one aphid visit or less to each plant in a week (Fig. 5B, left chart), but even that event would occur only after week 110. However, it was enough to increase to two the number aphid visits that reach each a plant/week to allow viral infection to reach an endemic equilibrium that would be maintained in time. It was inferred that the visit of 1.10 aphids per plant and week was sufficient to maintain PVY in the simulated environment. With more frequent aphid visits, the percentage of infected plants in the equilibrium stage became higher, until the population reached the maximum possible percentage of infection (85.36% of infected plants in our simulation because of how the population turnover is defined). To get to this maximum it was necessary that the effective transmission efficiency achieved the value of one, something that in the simulation happened when around 4 aphids (3.847) visited each plant per week.

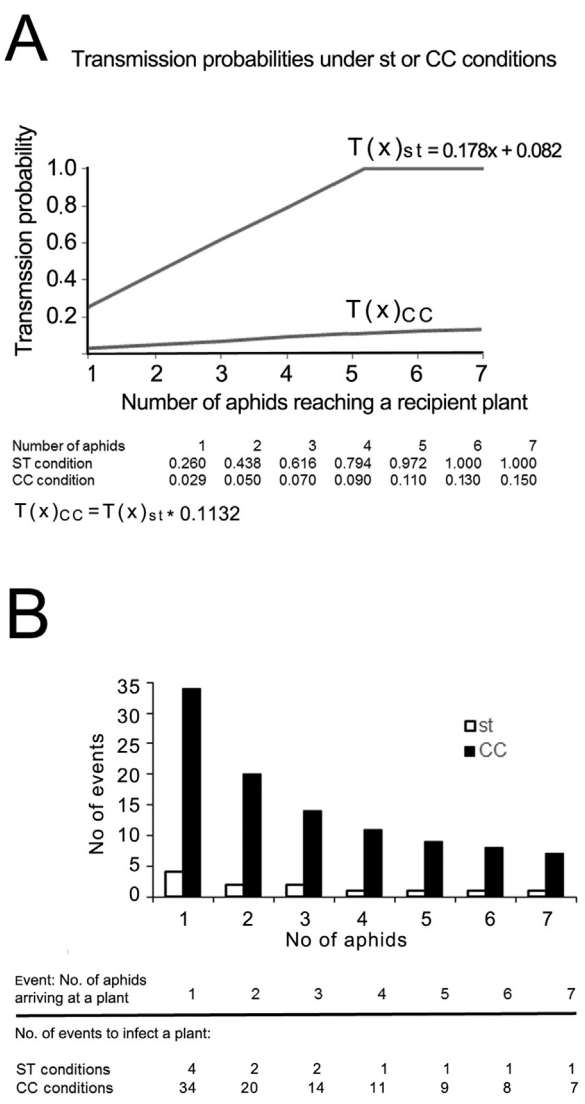

Fig. 4. Determination of the relative proportions of transmission under st or CC conditions. **A.,** the observed proportion of non-persistent transmission of infection by a given number (x) of aphids that fed on donor leaves from *Nicotiana benthamiana* plants infected by a potato virus Y (PVY) isolate that had been kept under standard (st) conditions of 25 °C and current atmospheric levels of CO_2 of ~405 parts-per-million [$T(x)_{st}$], or under conditions of elevate temperature and CO_2 levels [climate change-associated conditions (CC)] of 30 °C and 970 ppm of CO_2 [$T(x)_{CC}$], relative to the number of aphids that reached and probed on each recipient plantlet. The probability that an aphid transmits infection in a single probing of a recipient plantlet after having fed from leaves of plants that had been kept under either st or CC conditions was 0,26 (26%) or 0.029 (2.9%), respectively. **B.,** rounded-up estimate of the average number of events (first-time probings by aphids on recipient plantlets after having fed from leaves of infected plants kept under st or under CC conditions) that would be required to infect that plantlet, relative to the number of aphids that reached each recipient plantlet.

On the other hand, simulation of PVY dispersal and prevalence in time under CC conditions show that the virus disappeared from the environment when less than 10 aphids (9.881) visited each plant in a week (Fig. 5B, right chart). Only with values equal to or greater than 10 visiting aphids/plant/week infection became endemic, although with percentages of infection much lower than under st conditions. To reach the maximum possible of infected plants, the number of aphid visits/plant/week would have to be 34 (34.483), which is almost nine-times higher than required under st conditions. These results on prevalence are affected to an extent by the proportion of infected plants present at the start of the simulation (in Fig. 5B that proportion is 50%). An interactive model, in which different parameters used as input in the simulation could be modified, is provided as Supplementary Excel. File S1.

4. Discussion

In previous works we had studied how two environment abiotic parameters, temperature and CO_2 levels, influenced compatible infections of *N. benthamiana* plants by several RNA viruses. We had found significant decreases in the systemic titers of a PVY isolate when plants were kept under conditions of elevated temperature (30 °C), relative to plants kept under st conditions (del Toro et al., 2014). Those decreases were further exacerbated if plants were kept also simultaneously under elevated CO_2 levels (CC conditions; del Toro et al., 2017). Low viral titers were accompanied by much attenuated symptoms.

We wanted to investigate how viral dispersal by aphids would be affected by CC conditions in our patho-system PVY/*N. benthamiana* relative to st conditions. We determined experimentally the probabilities that our *M. persicae* biotype insects would transmit a PVY isolate between plants kept under the two different environments and investigated their relation with viral titers in donor leaves. We found strong positive correlation between titers and transmission probabilities (Figs. 1–4), in agreement to early observations relating titers and transmission of PVY in tobacco (De Bokx et al., 1978). Our results suggest that titers could be determinant in the differences in transmission probabilities observed from infected plants grown under st or CC environments.

It should be stressed that the results obtained under these specific controlled environments apply to our pathosystem, and that in other plant-virus-vector systems outcomes might be very different. It should also be acknowledged that the environment parameters studied here could influence transmission probabilities in additional ways, such as by altering the attractiveness or deterrence that plants exert on aphids (i.e., through color, leaf surface properties, plant volatile emissions, sap properties that could affect aphid behaviour: Fereres et al., 1999; Hodge and Powell, 2008; Dusi and Peters, 1999), or the behaviour of populations of insect vectors: it is known that elevated temperatures can have detrimental effects on the longevity and fecundity of adult females (Chiu et al., 2012), and that heat waves of around 30 °C can be harmful to aphid populations (Gillespie et al., 2002; Jeffs and Leather, 2014). Elevated ambient CO_2 levels also affect different parameters associated with the infestation of plants by aphids, including relative growth rate, colony growth rate, fecundity, development time, feeding efficiency, aphid colonization, susceptibility of host plant cultivars to aphids, interactions between aphids and plant endophytes, as well escape behaviour from aphid predators (Hughes and Bazzaz, 2001; Ryalls et al., 2015).

On the other hand, probabilities of transmission could also be influenced by physiological effects of environment conditions on recipient plants: it was recently reported that transmission of a CMV strain by *M. persicae* between pepper plants suffered a two-fold reduction when recipient plants had been pre-acclimated to elevated CO_2 levels before the transmission event when compared to non-pre-acclimated plantlets, exposed to elevated CO_2 levels only after the transmission event (Dáder et al., 2016). This suggests that in addition to changes in aphid feeding behaviour pre-exposure of plants to elevated CO_2 levels could reduce transmission probabilities even further through physiological alterations in the recipient plants (Dáder et al., 2016). However, in our experimental system we found no evidence of an effect of CC conditions on the abilities of recipient plants to become infected (Fig. 3).

With our data on transmission probabilities by individual insects we simulated the prevalence through time of infection by our PVY isolate on a homogeneous population of *N. benthamiana* plants under either environment scenario (Fig. 5). Most compatible plant viral infections

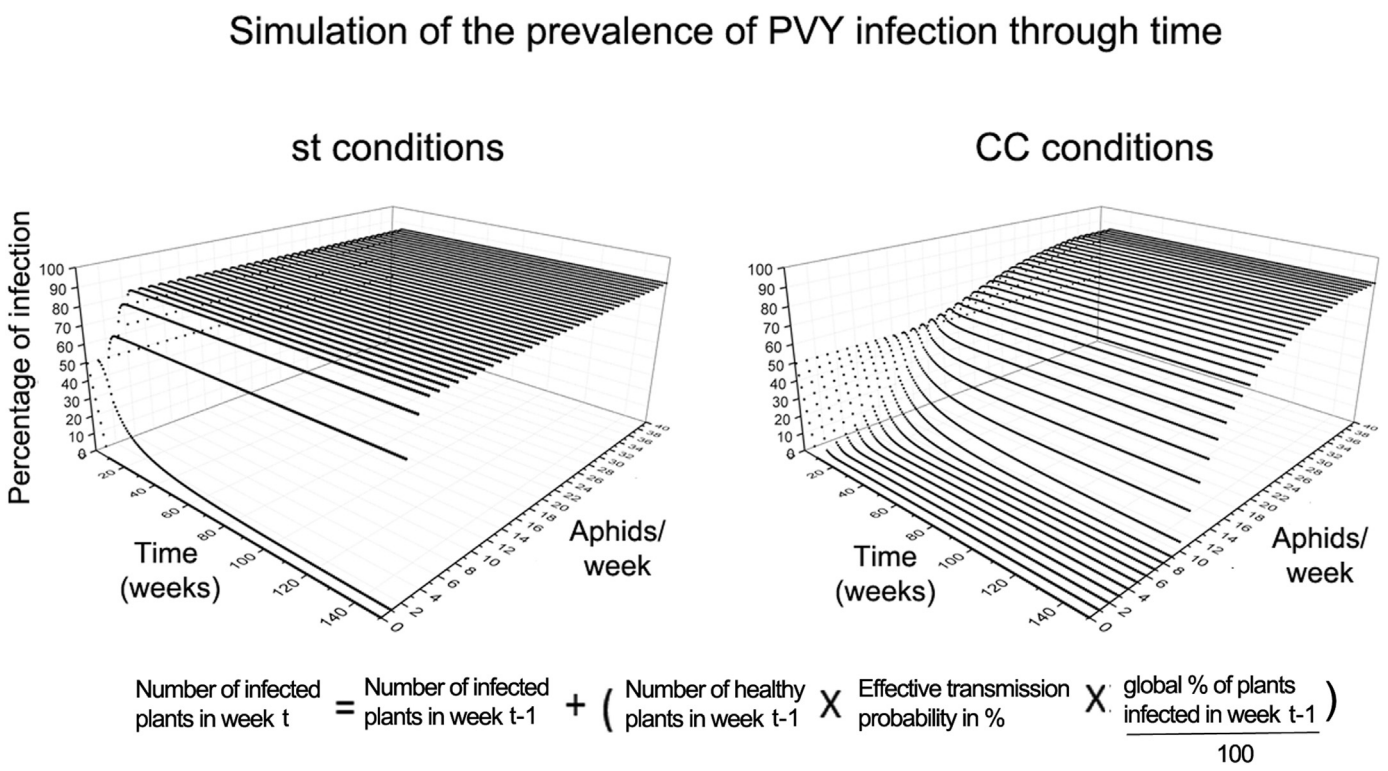

Fig. 5. Horizontal between-plants dispersal of PVY infection in *Nicotiana benthamiana* plants kept under either standard (st) conditions of 25 °C and ~404 parts-per-million (ppm) of ambient CO_2 levels, or under conditions of elevate temperature and CO_2 levels [climate change-associated conditions (CC)] of 30 °C and 970 ppm of CO_2, Simulation of the prevalence through time (in weeks) of infection in a population of plants with an arbitrary starting percentage of infection of 50%, under either st or CC conditions, left and right charts, respectively. Charts plot the percentage of infected plants (vertical axis) against time in weeks (x-axis), considering that a defined number of aphids visit and probe each plant in a week (y-axis). The simulation indicates that the visit of just over one aphid (1.104)/plant and week is sufficient to maintain PVY infection endemic under st conditions (left chart). By contrast, 10 (9.881) aphid visits/plant/week would be required under CC conditions (right chart). Under both environmental conditions, percentages of infected plants in the equilibrium stage rise with more aphid visits until they reach the maximum of infected plants (85.36% in our model), something that happens when around 4 or 34 aphids visited each plant/week, under st or CC conditions, respectively. Below the charts appears the formula used to obtain the simulated number of plants infected in each given week.

Table 1
Determination of the time that it takes for a plant to become systemically infected from the initial point of inoculation, under either standard (st) or climate change-associated (CC) conditions. No significant differences were observed.

	st	CC
Plant 1	6	5
Plant 2	5	6
Plant 3	6	6
Plant 4	6	NI
Plant 5	6	4
Plant 6	5	4
Plant 7	5	NI
Plant 8	6	6
Plant 9	5	6
No. of infected plants	9	7
Days to systemic infection	5.55	5.28

NI: not infected.
P > 0.05 (Mann-Whitney *U* test). No significant differences.

could be appropriately described by the SI (susceptible-infectious) model. In this model, the host plant that receives a viruliferous vector and is successfully infected becomes itself a source of infection for feeding vectors shortly after inoculation, differences in latency intervals are small and can be ignored, and plants remain infectious until death (Keeling and Rohani, 2007). In our simulation we assumed that plants homogeneously distributed in eight age groups and the number of new seedlings compensated for the number deaths leaving the population stable. (Table 1)

For simplicity, our analysis assumed: 1-that latency, or the length of time that it took a plant of any age to become systemically infected after successful inoculation by a viruliferous aphid, and thus to become itself a source for infection was the same under st or CC conditions; 2-that the abilities of aphids to feed and become viruliferous were the same under st or CC conditions, and that only or mainly virus titers in donor leaves affected their estimated probabilities to transmit the virus.

With regard to the 1st assumption, we experimentally measured the time that it took for same-age *N. benthamiana* plants to become infected systemically with PVY from an initial point of mechanical inoculation under st or CC conditions, and found that there were no significant differences (5.55 vs 5.28 days, respectively. Mann Whitney *U*-test $p > 0.05$; Fig. 5A). Thus, interestingly in our system CC conditions though affecting the levels of systemic virus and the symptoms induced, did not slow the long-distance movement of the virus. In other systems such as when the potyvirus beet mosaic virus infects sugar beet plants or in infections by PVY of potatoes, elevated temperatures affected the length of the latency period before plants became systemically infected (Dusi and Peters, 1999; Choi et al., 2017, respectively).

With regard to the 2nd assumption, in our transmission experiments we used donor leaves and recipient plantlets grown under different environment conditions, but transmission events were performed under st conditions. However, a recent work that used the same clonal population of *M. persicae* as the one used in this work and also *N. benthamiana* plants reported differences in transmission probabilities by aphids that fed under different temperatures (Chung et al., 2016). That work tested the potyvirus potato virus A (PVA) and a Korean strain of PVY that was different to ours and found that probabilities by individual aphids to transmit PVA or the korean isolate of PVY decreased

both significantly ~60% when they fed at 30 °C relative to when they fed at 25 °C (Chung et al., 2016). However, the insect colony was reared under st conditions and insects were thus not adapted to 30 °C. We did not incorporate those results into our simulation, but if we had extrapolated them into our system, transmission probabilities under CC conditions would have been ($0.029 \times 0.4 = 0.0116$), having a further negative impact on the simulated prevalence of infection under CC conditions.

An interactive algorithm where inputs can be modified in the simulation is provided in Supplemental material S1. In the simulation with an initial 50% of infected plants we found that under st conditions PVY infection remained in the population even when the number of visiting aphids that visit each plant per week was as low as 2. However, under CC conditions the number of aphid visits/plant/week would have increase several-fold for the prevalence of infection to remain comparable to that simulated under st conditions (Fig. 5B).

In conclusion, we have shown that in our experimental pathosystem conditions of elevated ambient temperature and CO_2 levels decreased probabilities of transmission of viral infection by aphids and this also correlated with reduced viral titers in the donor leaves. Reduced transmission was detrimental to the simulated prevalence of infection in the environment. As mentioned, work by others suggests that those probabilities of transmission could decrease even further under CC conditions because of additional effects on insect feeding behaviour (Chung et al., 2016). Unless other factors compensate for those losses, or selective pressure on the variability of the isolate increases its transmissibility/titers, our data suggest that extended warm episodes and permanent elevated levels of CO_2 associated to climate change could challenge the prevalence of infections by some populations of non-persistently transmitted potyviruses.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.virol.2019.02.001.

References

Aguilar, E., Allende, L., del Toro, F.J., Chung, B.-N., Canto, T., Tenllado, F., 2015. Effects of elevated CO_2 and temperature on pathogenicity determinants and virulence of Potato virus X/potyvirus-associated synergism. Mol. Plant-Microbe Interact. 28, 1364–1373.

Ammar, E.D., Jarlfors, U., Pirone, T.P., 1994. Association of potyvirus helper component protein with virions and the cuticle lining the maxillary food canal and foregut of an aphid vector. Phytopathology 84, 1054–1060.

Atreya, P.-L., Atreya, C.D., Pirone, T.P., 1991. Amino acid substitutions in the coat protein result in loss of insect transmissibility of a plant virus. Proc. Natl. Acad. Sci. USA 88, 7887–7891.

Atreya, C.D., Atreya, P.L., Thornbury, D.W., Pirone, T.P., 1992. Site-directed mutations in the potyvirus HC-PRO gene affect helper component activity, virus accumulation, and symptom expression in infected tobacco plants. Virology 191, 106–111.

Banik, M.T., Zitter, T.A., 1990. Determination of Cucumber mosaic virus titer in muskmelon by enzyme-linked immunosorbent assay and correlation with aphid transmission. Plant Dis. 74, 857–859.

Barker, H., McGeachy, K.D., Toplak, N., Gruden, K., Žel, J., Browning, I., 2009. Comparison of genome sequence of PVY isolates with biological properties. Am. J. Potato Res. 86, 227–238.

Baulcombe, D.C., Manoussopoulos, I.N., Roberts, I.M., Harrison, B.D., 1993. Signal for potyvirus-dependent aphid transmission of potato aucuba mosaic virus and the effect of its transfer to Potato virus X. J. Gen. Virol. 74, 1245–1253.

Blanc, S., Ammar, E.D., García-Lampasona, S., Dolja, V.V., Llave, C., Baker, J., Pirone, T.P., 1998. Mutations in the potyvirus helper component protein: effects on interactions with virions and aphid stylets. J. Gen. Virol. 79, 3119–3122.

Brault, V., Uzest, M., Monsion, B., Jacquot, E., Blanc, S., 2010. Aphids as transport devices for plant viruses. C. R. Biol. 333, 524–538.

Canto, T., López-Moya, J.J., Serra-Yoldi, M.T., Díaz-Ruíz, J.R., López-Abella, D., 1995. Different helper component mutations associated with lack of aphid transmissibility in two isolates of Potato virus Y. Phytopathology 85, 1519–1524.

Chiu, M.-C., Chen, Y.-H., Kuo, M.-H., 2012. The effect of experimental warming on a low-latitude aphid, Myzus varians. Entomol. Exp. Appl. 142, 216–222.

Choi, K.S., del Toro, F.J., Tenllado, F., Canto, T., Chung, B.-N., 2017. A model to explain temperature dependent systemic infection of potato plants by Potato virus Y. Plant Pathol. J. 33, 206–211.

Chung, B.-N., Canto, T., Tenllado, F., Choi, K.S., Joa, J.H., Ahn, J.J., 2016. The effects of high temperature on infection by Potato virus Y, Potato virus A, and Potato leafroll virus. Plant Pathol. J. 32, 321–328.

Dáder, B., Fereres, A., Moreno, A., Trębicki, P., 2016. Elevated CO_2 impacts bell pepper growth with consequences to *Myzus persicae* life history, feeding behaviour and virus transmission ability. Sci. Rep. 6, 19120.

De Bokx, J.A., Piron, D.G.M., 1977. Effect of temperature on symptom expression and relative concentration of Potato viruses S and M in cv. Eerstel. Med. Fak. Landbouw. Rijksuniv. Gent 42/2, 1977.

De Bokx, J.A., Van Hoof, H.A., Piron, P.G.M., 1978. Relation between concentration of Potato virus Y^N and its availability to Myzus persicae. Neth. J. Plant Pathol. 84, 95–100.

del Toro, F.J., Tenllado, F., Chung, B.-N., Canto, T., 2014. A procedure for the transient expression of genes by agroinfiltration above the permissive threshold, to study temperature-sensitive processes in plant-pathogen interactions. Mol. Plant Pathol. 15, 848–857.

del Toro, F.J., Aguilar, E., Hernández-Walias, F.J., Tenllado, F., Chung, B.-N., Canto, T., 2015. High temperature, high ambient CO_2 affect the interactions between three positive-sense RNA viruses and a compatible host differentially, but not their silencing suppression efficiencies. PLoS One 10, e0136062. https://doi.org/10.1371/journal.pone.0136062.

del Toro, F.J., Mencía, E., Aguilar, E., Tenllado, F., Canto, T., 2018. HCPro-mediated transmission by aphids of purified virions does not require its silencing suppression function and correlates with its ability to coat cell microtubules in loss-of-function mutant studies. Virology 525, 10–18.

Dusi, A.N., Peters, D., 1999. *Beet mosaic virus*: its Vector and Host relationships. 1999. J. Phytopatol. 147, 193–298.

Escriu, F., Perry, K., García-Arena, l.F., 2000. Transmissibility of Cucumber mosaic virus by *Aphis gossypii* correlates with viral accumulation and is affected by the presence of its satellite RNA. Phytopathology 90, 1068–1072.

Fereres, A., Kampmeier, G.E., Irwin, M.E., 1999. Aphid attraction and preference for soybean and pepper plants infected with Potyviridae. Ann. Entomol. Soc. Am. 92, 542–548.

Fereres, A., Moreno, A., 2009. Behavioural aspects influencing plant virus transmission by homopteran insects. Virus Res. 141, 158–168.

Fernández-Calvino, L., Goytia, E., López-Abella, D., Giner, A., Urizarna, M., Vilaplana, Ll, López-Moya, J.J., 2010. The helper-component protease transmission factor of tobacco etch potyvirus binds specifically to an aphid ribosomal protein homologous to the laminin receptor precursor. J. Gen. Virol. 91, 2862–2873.

Froissart, R., Doumayrou, J., Vuillaume, F., Alizon, S., Michalakis, Y., 2010. The virulence-transmission trade-off in vector-borne plant viruses: a review of (non-)existing studies. Philos. Trans. R. Soc. B-Biol. Sci. 365, 1907–1918.

Gera, A., Loebenstein, G., Raccah, B., 1979. Protein coats of two strains of Cucumber mosaic virus affect transmission by *Aphis gossypii*. Phytopathology 69, 396–399.

Gillespie, T., Boevink, P., Haupt, S., Roberts, A.G., Toth, R., Valentine, T., Chapman, S., Oparka, K.J., 2002. Functional analysis of a DNA-shuffled movement protein reveals that microtubules are dispensable for the cell-to-cell movement of tobacco mosaic virus. Plant Cell 14, 1207–1222.

Hodge, S., Powell, G., 2008. Do plant viruses facilitate their aphid vectors by inducing symptoms that alter behavior and performance? Environ. Entomol. 37, 1573–1581.

Huet, H., Gal-On, A., Meir, E., Lecoq, H., Raccah, B., 1994. Mutations in the helper component protease of zucchini yellow mosaic virus affect its ability to mediate aphid transmissibility. J. Gen. Virol. 75, 1407–1414.

Hughes, L., Bazzaz, F.A., 2001. Effects of elevated CO_2 on five plant-aphid interactions. Entomol. Exp. Appl. 99, 87–96.

Hull, R., 2002. Disease symptoms and host range. In: Matthew's Plant Virology, Fourth edition. Elsevier, San Diego, California, USA, pp. 47–74.

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (eds.), IPCC, Geneva, Switzerland, 151 pp.

Jeffs, C.T., Leather, S.R., 2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid, *Sitobion avenae*. Entomol. Exp. Appl. 150, 240–249.

Keeling, M.J., Rohani, P., 2007. Modeling Infectious Diseases in Humans and Animals (ISBN 9780691116174. 408 pp). Princeton Univ. Press.

Llave, C., Martínez, B., Díaz-Ruíz, J.R., López-Abella, D., 1999. Helper component mutations in nonconserved residues associated with aphid transmission efficiency of a pepper isolate of Potato virus Y. Phytopathology 89, 1176–1181.

Llave, C., Martínez, B., Díaz-Ruíz, J.R., López-Abella, D., 2002. Amino acid substitutions within the Cys-rich domain of the tobacco etch potyvirus HC-Pro result in loss of transmissibility by aphids. Arch. Virol. 147, 2365–2375.

López-Moya, J.J., Wang, R.Y., Pirone, T.P., 1999. Context of the coat protein DAG motif affects potyvirus transmissibility by aphids. J. Gen. Virol. 80, 3281–3288.

Martín, B., Collar, J.L., Tjallingii, W.F., Fereres, A., 1997. Intracellular ingestion and

salivation by aphids may cause the acquisition and inoculation of nonpersistently transmitted plant viruses. J. Gen. Virol. 78, 2701–2705.
Matros, A., Amme, S., Kettig, B., Buck-Sorlin, G.H., Sonnewald, U., Mock, H.-P., 2006. Growth at elevated CO_2 concentrations leads to modified profiles of secondary metabolites in tobacco cv. SamsunNN and to increased resistance against infection with Potato virus Y. Plant Cell Environ. 29, 126–137.
Moury, B., Fabre, F., Senoussi, R., 2007. Estimation of the number of virus particles transmitted by an insect vector. Proc. Natl. Acad. Sci. USA 104, 17891–17896.
Normand, R.A., Pirone, T.P., 1968. Differential transmission of strains of cucumber mosaic virus by aphids. Virology 36, 538–544.
Peng, Y.-H., Kadoury, D., Gal-On, A., Huet, H., Wang, Y., Raccah, B., 1998. Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: effects on aphid transmission and binding to purified virions. J. Gen. Virol. 79, 897–904.
Pirone, T.P., Megahed, E., 1966. Aphid transmissibility of some purified viruses and viral RNAs. Virology 30, 630–637.
Roossinck, M., 1991. Temperature-sensitive replication of cucumber mosaic virus in muskmelon (*Cucumis melo* cv. Iroquois), maps to RNA 1 of a slow strain. J. Gen. Virol. 72, 1747–1750.
Powell, G., 2005. Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. J. Gen. Virol. 86, 469–472.
Ryalls, J.M.W., Moore, B.D., Riegler, M., Gherlenda, A.N., Johnson, S.N., 2015. Amino acid-mediated impacts of elevated carbon dioxide and simulated root herbivory on aphids are neutralized by increased air temperatures. J. Exp. Bot. 66, 613–623.
Szittya, G., Silhavy, D., Molnár, A., Havelda, Z., Lovas, Á., Lakatos, L., Bánfalvi, S., Burgyán, J., 2003. Low temperature inhibits RNA silencing-mediated defence by the control of siRNA generation. EMBO J. 22, 633–640.
Trębicki, P., Vandegeer, R.K., Bosque-Pérez, N.A., Powell, K.S., Dáder, B., Freeman, A.J., Yen, A.L., Fitzgerald, G.J., Luck, J.E., 2016. Virus infection mediates the effects of elevated CO_2 on plants and vectors. Sci. Rep. 6, 22785.
Uzest, M., Gargani, D., Drucker, M., Hébrard, E., Garzo, E., Candresse, T., Fereres, A., Blanc, S., 2007. A protein key to plant virus transmission at the tip of the insect vector stylet. Proc. Natl. Acad. Sci. USA 104, 17959–17964.
Uzest, M., Gargani, D., Dombrovsky, A., Cazevieille, C., Cot, D., Blanc, S., 2010. The "acrostyle": a newly described anatomical structure in aphid stylets. Arthropod Struct. Dev. 39, 221–229.
van Munster, M., Yvon, M., Vile, D., Dáder, B., Fereres, A., Blanc, S., 2017. Water deficit enhances the transmission of plant viruses by insect vectors. PLoS One 12 (5), e0174398.
Watson, M.A., Roberts, F.M., 1939. A comparative study of the transmission of Hyoscyamus virus 3, Potato virus Y and Cucumber virus 1 by the vectors Myzus persicae (Sulz), M. circumflexus (Buckton), and Macrosiphum gei (Koch). Proc. Roy. Soc. B127, 543–576.
Whitfield, A.E., Falk, B.W., Rotenberg, D., 2018. Insect vector-mediated transmission of plant viruses. Virology 479–480, 278–289.
Ye, L., Fu, X., Ge, F., 2010. Elevated CO_2 aleviates damage from Potato virus Y infection in tobacco plants. Plant Sci. 179, 219–224.
Zhang, S., Li, X., Sun, Z., Shao, S., Hu, L., Ye, M., Zhou, Y., Xia, X., Yu, J., Shi, K., 2015. Antagonism between phytohormone signaling underlies the variation in disease susceptibility of tomato plants under elevated CO_2. J. Exp. Bot. https://doi.org/10.1093/jxb/eru538.