Plant Pathology (2019) **68**, 72–84 Doi: 10.1111/ppa.12920

Temporal and spatial progress of the diseases caused by the crinivirus tomato chlorosis virus and the begomovirus tomato severe rugose virus in tomatoes in Brazil

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Efficient management of whitefly-borne diseases remains a challenge due to the lack of a comprehensive understanding of their epidemiology, particularly of the diseases tomato golden mosaic and tomato yellowing. Here, by monitoring 16 plots in four commercial fields, the temporal and spatial distribution of these two diseases were studied in tomato fields in Brazil. In the experimental plots these diseases were caused by tomato severe rugose virus (ToSRV) and tomato chlorosis virus (ToCV), respectively. The incidence of each virus was similar in the plots within a field but varied greatly among fields. Plants with symptoms for both diseases were randomly distributed in three of four spatial analyses. The curves representing the progress of both diseases were similar and contained small fluctuations, indicating that the spread of both viruses was similar under field conditions. In transmission experiments of ToSRV and ToCV by *Bemisia tabaci* MEAM1 (former biotype B), these viruses had a similar transmission rate in single or mixed infections. It was then shown that primary and secondary spread of ToCV were not efficiently controlled by insecticide applications. Finally, in a typical monomolecular model of disease progress, simulation of the primary dissemination of ToSRV and ToCV showed that infected plants were predominantly randomly distributed. It is concluded that, although the manner of vector transmission differs between ToSRV (persistent) and ToCV (semipersistent), the main dispersal mechanisms are most probably similar for these two diseases: primary spread is the predominant mechanism, and epidemics of these diseases have been caused by several influxes of viruliferous whiteflies.

Keywords: disease management, epidemiology, *Solanum lycopersicum*, whitefly

Introduction

Tomato golden mosaic disease, which is caused by complexes of begomoviruses, is the major viral disease that affects tomato production in Brazil (Inoue-Nagata *et al.*, 2016). Many begomoviruses infect tomato plants in Brazil, but tomato severe rugose virus (ToSRV) is predominant in most tomato production areas (Inoue-Nagata *et al.*, 2016). The begomoviruses belong to the family *Geminiviridae* and have a mono- or bipartite genome composed of circular single-stranded DNA encapsidated in twinned particles and are transmitted by cryptic species of the whitefly *Bemisia tabaci*.

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Published online 4 September 2018

Tomato yellowing disease was first reported in Brazil in 2008 in the state of São Paulo (Barbosa *et al.*, 2008). This disease is caused by the crinivirus tomato chlorosis virus (ToCV), which is also transmitted by whiteflies. This disease has rapidly spread across the main tomato production areas in Brazil (Barbosa *et al.*, 2011; Macedo *et al.*, 2014). ToCV is the only crinivirus that has been reported in tomatoes in Brazil (Barbosa *et al.*, 2008, 2011), and little genetic variability has been found among isolates (Albuquerque *et al.*, 2013). Criniviruses belong to the family *Closteroviridae*, and their genomes are composed of two single-stranded positive-sense RNA molecules.

Begomoviruses are persistently transmitted by species of the *B. tabaci* complex (Rosen *et al.*, 2015), and ToCV is semipersistently transmitted by *B. tabaci* complexes as well as *Trialeurodes vaporariorum* and *T. abutiloneus* (Wisler *et al.*, 1998). The heavy infestation of *B. tabaci* MEAM1 (Middle East Asia Minor 1 species, also known as biotype B) in the agricultural areas of Brazil, the presence of large numbers of alternative hosts for the viruses in the field, and the difficulty in managing the insect vector *B. tabaci* are believed to be responsible for the high incidence of these whitefly-transmitted viruses.

The control of golden mosaic disease usually relies on the use of resistant tomato cultivars and frequent applications of insecticides for vector control (Inoue-Nagata *et al.*, 2016), while chemical spraying is the major control method used for the yellowing disease, due to the absence of resistant cultivars (Mansilla-Córdova *et al.*, 2018). Even though tomato growers in Brazil usually spray insecticides to combat whiteflies two or three times per week to prevent the spread of both viruses, the high incidence of the diseases caused by ToSRV and ToCV demonstrates that this strategy has low efficacy (Inoue-Nagata *et al.*, 2016). Recently, the efficiency of insecticide (cyantraniliprole, spiromesifen, thiamethoxam and cartap) applications on the control of whiteflies was evaluated under simulated primary and secondary dispersions of ToSRV by *B. tabaci* MEAM1 (Gouvêa *et al.*, 2017). The authors concluded that no insecticide was effective at controlling the primary dispersion of ToSRV by *B. tabaci*. In contrast, cyantraniliprole (applied as a root drench and foliar spray) and cartap (spray) were effective at controlling the secondary spread of the virus, as the infection rate was reduced by 81–94.5% compared to that of the untreated controls.

Based upon a temporal and spatial epidemiological study performed on tomato production fields in Brazil, it was recently proposed that golden mosaic disease in tomatoes is disseminated by three mechanisms: one with a random distribution (primary) and two with an aggregated distribution, the classical secondary spread and a kind of false secondary spread (Macedo *et al.*, 2017). In the last mechanism, viruliferous whiteflies enter from outside of the field, after which they feed on and inoculate multiple nearby plants with the begomovirus, resulting in an aggregated distribution pattern. The results of this previous study strongly indicate that primary spread is the most important mechanism for the spread of begomovirus in the Brazilian tomato cultivation system (Macedo *et al.*, 2017). However, little is known about the spread of the yellowing disease in tomatoes, particularly under tropical agricultural conditions. The objective of this study was therefore to compare the temporal and spatial progress of the diseases golden mosaic and yellowing in the tomato production system of Brazil both in a controlled environment (screen cages) and in open fields. This study was complemented by a thorough study on the transmission aspects; the efficiency of adult whiteflies in transmitting both viruses individually or simultaneously was evaluated, and the effects of five insecticides on the control of simulated primary and secondary spread of ToCV were also investigated.

Materials and methods

Monitoring disease spread in the field

Location, experimental design and data collection

Experimental plots were established in fields in four areas of tomato production in central Brazil; the tomatoes of these areas were grown for fresh markets. Two fields were in the state of Goiás, at Goianápolis (16°30′21.3″S, 47°43′18.3″W; 984 m a.s.l.) and Anápolis (16°31′48.9″S, 48°48′14.4″W; 968 m a.s.l.), and two fields were in the Federal District, at Boa Esperança (15°49′23.14″S, 48°14′39.3″W; 950 m a.s.l.) and Taquara (15°39′31.11″S, 47°33′12.8″W; 1110 m a.s.l.). The study was conducted from September 2012 to October 2014. Virus- and whitefly-free tomato transplants of cultivars resistant to begomovirus infection were used in all fields: Dominador (TopSeed) in Goianápolis, Anápolis and Taquara, as well as Predador (TopSeed) in Boa Esperança. By carrying the *Ty-1* resistance gene, both Dominador and Predador are moderately resistant to begomovirus infection; however, Dominador is moderately resistant, and Predador is highly susceptible to ToCV infection (Mansilla-Córdova *et al.*, 2018). In these plants, the infection rate to Brazilian begomoviruses (e.g. ToSRV) is relatively low, and when infected, the plants exhibit relatively mild symptoms (authors' unpublished observations). These transplants were produced in nurseries under high-quality control. The transplants were manually planted *c.* 30 days after sowing at a spacing of 1.5 × 0.7 m and were trained on cross-shaped bamboo stakes. The transplants were randomly sampled after transplanting and tested for begomovirus and crinivirus infections as described below. The plants were irrigated, treated with pesticides, and fertilized following the standard cultivation procedures of each grower.

A total of 16 plots were established in the fields: eight in Goiás and eight in the Federal District (four plots per location). Each plot consisted of 225 plants (15 × 15), and each plant was visually evaluated weekly for symptoms of golden mosaic and yellowing diseases. Symptoms exhibited by infected tomato plants in the fields were extensively evaluated and correlated with viral detection tests. It was confirmed that plants infected by a begomovirus and plants infected by a crinivirus could be differentiated with a high degree of confidence. Plants with chlorotic spots, interveinal chlorosis, leaf curling, rugosity in the upper leaves and stunting were diagnosed as infected by a begomovirus (Fig. 1a,b), and plants with leaf rolling and interveinal chlorotic areas mainly in the basal and middle leaves were diagnosed as infected by a crinivirus (Fig. 1c–e). Plants with symptoms were collected from all fields to confirm the visual diagnoses. These two viruses were the major phytosanitary problems in all 16 plots. Plants with tospovirus (*Groundnut ringspot virus*) infection were scattered at a low incidence (<1%) in the plots, and in a few fields bacterial spot (*Xanthomonas* spp.) and South American tomato moth (*Tuta absoluta*) problems were observed.

Detection and identification of viral species

The nucleic acids were extracted, and the presence of ToSRV or ToCV was tested by PCR or reverse transcription (RT)-PCR, respectively, with specific primers as described below. A total of 317 samples from plants with symptoms were collected to confirm the symptom evaluations: 130 exhibited symptoms typical of ToSRV infection, 140 exhibited symptoms typical of ToCV infection, and 47 exhibited symptoms of both diseases. Total DNA (Doyle & Doyle, 1990) or RNA (TRIzol; Invitrogen) was extracted from each sample. For detection of begomoviruses, total DNA was amplified by PCR with degenerate begomovirus primers PAR1c496/PAL1v1978, generating a DNA fragment of approximately *c.* 1.1 kb (Rojas *et al.*, 1993). Positive samples were then used to detect ToSRV using a new PCR that amplified a *c.* 0.8 kb fragment specific to ToSRV (Fernandes *et al.*, 2010). For detection of ToCV, total RNA was used for RT-PCR with the ToCV-specific primer pair ToC-5/ToC-6, which amplified a 463 bp DNA fragment (Dovas *et al.*, 2002). For each area of

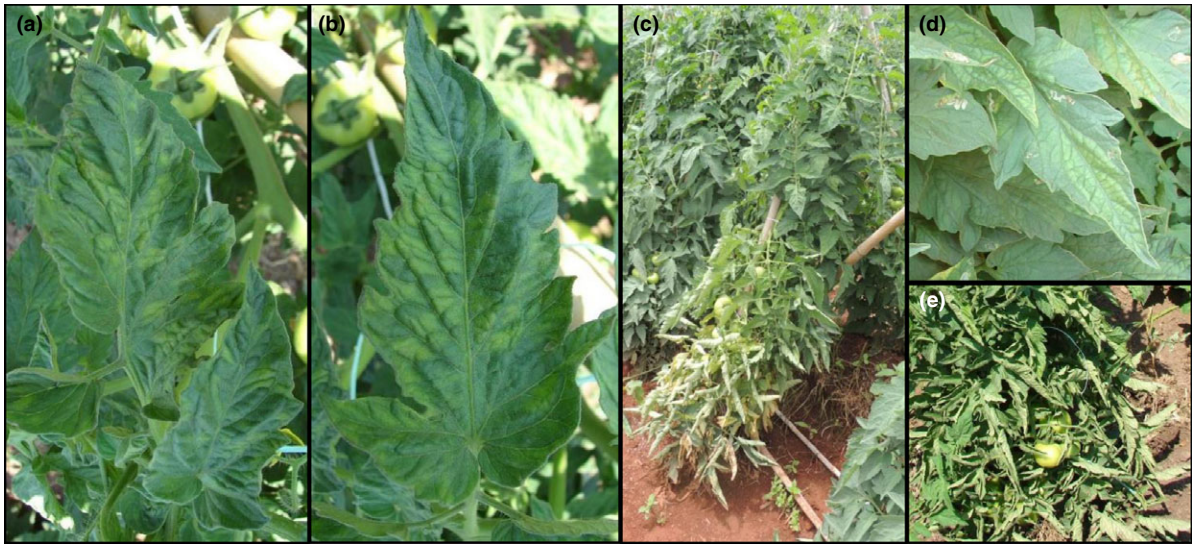

Figure 1 Tomato plants infected with tomato severe rugose virus (ToSRV), with symptoms of interveinal chlorosis and leaf curling in young leaves (a, b). Plants infected with tomato chlorosis virus (ToCV), with symptoms of interveinal chlorosis (d) and leaf curling in old leaves (c, e). [Colour figure can be viewed at wileyonlinelibrary.com].

the four locations, five PCR-positive samples for ToSRV (total of 20 samples) and for ToCV (total of 20 samples) were randomly selected and directly sequenced with primers PAR1c496 and ToC-5, respectively.

Temporal analysis

Maps representing the 16 plots were generated for each evaluation and used in temporal and spatial analyses. Plants with symptoms were marked on a 15 × 15 grid at the exact position in each evaluation. Two quadrat sizes were used for the spatial analysis: 2 × 3 (two plants in three rows) and 3 × 2 (three plants in two rows). The presence of symptoms for the 2 × 3 quadrats was evaluated in the first two plants of the first three rows, and then the next group of 2 × 3 plants was evaluated for the same rows; this pattern continued until the end of the 15 × 15 grid. For the 3 × 2 quadrats, three plants were evaluated in each of two rows until the end of the grid.

The incidence of tomato plants with symptoms (*P*) was assessed weekly in all 16 plots by visual inspection. The *P* in each plot was determined for each evaluation as described by Madden & Hughes (1995):

$$P = \sum(X_i)/nN$$

where X_i is the total number of plants with symptoms in each quadrat, n is the number of plants per quadrat, and N is the total number of quadrats in each plot.

Curves representing the progress of disease were developed for all plots, but no statistical model was adjusted. The area under the disease progress curve (AUDPC) was calculated by trapezoidal interception as described by Campbell & Madden (1990):

$$\text{AUDPC} = \sum((y_i + y_{i+1})/2)(t_{i+1} - t_i)$$

where y_i and y_{i+1} are the incidences in two consecutive assessments at times t_{i+1} and t_i, respectively.

Spatial analysis

The spatial pattern of the distribution of plants with symptoms was studied based on an ordinary run analysis, which is a nonparametric and simple test used to detect nonrandomness of plants with symptoms within rows (Madden *et al.*, 1982), the dispersion index, and the binary power law, as well as a dynamic analysis of disease foci. Binary maps (including plants with and without symptoms) were first generated. Each new plant with symptoms was plotted on the map in successive assessments. The quadrats of 2 × 3 and 3 × 2 were chosen to determine the spatial parameters of the plants with symptoms.

Ordinary run analysis

Rows were combined to form a single row with a length equal to the total number of plants in each plot. A row was considered to have a nonrandom sequence of diseased and symptomless plants if the standardized variable Z_u was < -1.64 (Campbell & Madden, 1990). An ordinary run test was performed for all plots.

*Dispersion index (*D*)*

D was calculated for the 2 × 3 and 3 × 2 quadrats from the observed (V_{obs}) and binomial (V_{bin}) variances (Madden & Hughes, 1995) as:

$$D = V_{obs}/V_{bin}$$

$$V_{obs} = \sum(x_i - Pn)2/n2(N-1)$$

$$V_{bin} = P(P-1)/n$$

where x_i is the total number of plants with symptoms in each quadrat, P is the incidence, n is the number of plants per quadrat, and N is the total number of quadrats in each plot.

The significance of *D* was determined by the chi-square test at 5% probability. A *D*-value of 1 was considered a random distribution (null hypothesis), and values that differed significantly from 1 were considered aggregates.

Binary power law

The application of the binary power law was used to characterize the linear relationship between the logarithms of V_{bin} and V_{obs} within each plot (Madden & Hughes, 1995) as:

$$\log(V_{bin}) = \log(A) + b\log(V_{obs})$$

where A and b are parameters.

Regressions were performed using STATISTICA v. 6.0 (StatSoft). The logarithm of binomial variances was considered the independent variable, whereas the logarithms of variances for the maps of each plot were considered dependent variables. The significance of the relationships (linear regression) between log (V_{bin}) and log (V_{obs}) was determined by an F-test at 5% probability. The goodness of fit of the data was determined by calculating the coefficient of determination (R^2) and the distribution patterns of the residues (Madden & Hughes, 1995).

The equality of the parameters $b = 1$ and $\log(A) = 0$ was evaluated by a t-test at 5% probability (Madden & Hughes, 1995) using the estimates of the random parameters and their standard errors. When $b = 1$ and $\log(A) = 0$, it was concluded that the aggregation followed a random distribution. When $b = 1$ and $\log(A) > 0$, the aggregation level was independent of the incidence. When $b > 1$ and $\log(A) > 0$, the aggregation varied with the incidence.

Dynamic analysis of disease foci (DADF)

The focus of a DADF is an area of concentration of infected plants that can become primary sites of infection or is coincident with areas originally that are both favourable to the establishment of the disease and that tend to influence the pattern of subsequent dissemination (Laranjeira *et al.*, 2004). The DADF was determined using the cumulative maps of each plot for each evaluation. Plants with symptoms were in the same focus (disease cluster) if they were immediately adjacent to plants with symptoms in terms of horizontal, vertical or diagonal proximity (Laranjeira *et al.*, 2004). Maps were constructed by plotting the number of foci related to the disease incidence for all data sets and fitted to a generalized β function (Hau & Kranz, 1990) as:

$$Y = b_1(x)^{b_2} \times (1 - x)^{b_3}$$

where Y is the number of foci per 225 plants, x is the disease incidence, and b_1, b_2 and b_3 are the calculated regression constants.

Association between golden mosaic and yellowing diseases

The association between the golden mosaic and yellowing diseases was quantified by the Jaccard index (J) (Turechek & Madden, 2000). The Jaccard index was calculated for each plot as:

$$J = a/(a + b + c)$$

where a is the number of plants infected by both viruses, b is the number of plants infected with virus one (1), and c is the number of plants infected with virus two (2). The index ranges from 0 to 1. When J is closer to 1, the association between the viruses is higher, and when J is closer to 0, the association between the viruses is lower.

Estimating whitefly infestations

The whitefly infestation was monitored weekly by placing four 12 × 12 cm yellow sticky card traps (Bio Controle) in each plot. These traps are sticky on both sides. The traps were placed at plant height, fixed on aluminium wires, and left in the field for 7 days. Adult whiteflies on both sides of the traps were counted with a stereomicroscope. The whitefly infestation was quantified by dividing the number of adult whiteflies from all four traps per plot by four to obtain the average number of adult whiteflies captured per trap per week in the plots. A regression analysis was performed to verify the relationship between the whitefly infestation and the incidence of golden mosaic and yellowing diseases.

Experiments under controlled conditions

Maintenance of viruses, whiteflies and plants

The isolates ToSRV-SP01, ToSRV-1164 (Macedo *et al.*, 2015) and ToCV-Br (Albuquerque *et al.*, 2013) were used for inoculations of tomato plants. The viruses were maintained in the tomato plants by whitefly transmission in insect-proof greenhouses.

Adults of *B. tabaci* MEAM1 were maintained for several generations on cabbage or kale plants (*Brassica oleracea*), both of which are nonhost species of both ToSRV and ToCV, and maintained in insect-proof greenhouses. The insects were identified as *B. tabaci* MEAM1 by methods based on PCR amplification of the mitochondrial cytochrome oxidase I gene followed by enzymatic digestion (Frohlich *et al.*, 1999; Bosco *et al.*, 2006). Inoculation was performed on tomato cv. Kada (transmission and insecticide evaluation experiments) or cv. Santa Clara (experiment on temporal and spatial progress of disease) plants that had two or three developed true leaves (*c.* 30 days after seeding).

Transmission of ToSRV and ToCV with B. tabaci *MEAM1*

Virus-free adults of *B. tabaci* MEAM1 were separately confined on tomato leaves systemically infected with (i) ToCV, (ii) ToSRV (isolate ToSRV-SP01), and (iii) both viruses. After an acquisition access period (AAP) of 24 h, the insects were confined in cages of 1.5 cm in diameter by 1 cm in height and then placed underneath a leaf of a healthy tomato test plant. Only one individual insect was confined per plant for an inoculation access period (IAP) of 24 h. After inoculation, the cages and whiteflies were manually removed. Noninoculated tomato plants were used as controls, and all plants were maintained in the greenhouse. Approximately 40 days after inoculation, leaf samples were collected from each plant for detection of ToSRV and ToCV by PCR and RT-PCR, respectively, as described previously. This experiment was performed in four independent trials.

Evaluation of insecticides

The following formulations of insecticides were used in the tests: cyantraniliprole root drench (Verimark), pymetrozine (Chess), acetamiprid (Mospilan), flupyradifurone (Sivanto) and cartap (Cartap), all of which were purchased at a local market. Tomato plants were treated with one of the following applications of formulated insecticides: cyantraniliprole root drenching at 2.5 mL L^{-1}, pymetrozine at 0.4 g L^{-1}, acetamiprid at 0.4 g L^{-1}, flupyradifurone at 2 mL L^{-1}, and cartap at 2.5 g L^{-1}. With the exception of the cyantraniliprole solution, which was applied to the soil (10 mL per plant), all insecticides were dissolved in

deionized water and subsequently sprayed onto tomato plants at the three-leaf stage until run-off occurred.

Two trials were performed to evaluate the efficiency of the insecticides for the control of the primary and secondary transmission of ToCV by *B. tabaci* MEAM1. In each trial, three treatments were tested: simulation of primary transmission (P); simulation of secondary transmission (S); and the respective controls for the primary and secondary transmission. The treatments were separated in individual wooden cages (0.7 × 1.2 × 1.7 m), which were covered with voile fabric.

To simulate primary spread, 25 healthy plants of tomato cv. Kada in separate cages were treated with each insecticide. After 48 h, 250 ToCV-viruliferous whiteflies (see above for the preparation of viruliferous *B. tabaci*) at an average of 10 insects per plant were released into the cage. Two rounds of insecticide spraying and the subsequent release of 250 viruliferous whiteflies occurred for each cage at 7-day intervals following the first release of the insects. Under the same conditions described above, 25 healthy tomato plants, which were sprayed with water, were placed in another cage and inoculated with ToCV-viruliferous *B. tabaci* (control of the primary transmission).

To simulate secondary spread, 25 healthy and three ToCV-infected plants of tomato cv. Kada were placed in a cage and treated with each insecticide. After 48 h, 250 virus-free adults of *B. tabaci* were released into each cage. Insecticide application and the subsequent release of virus-free insects occurred twice for each cage at an interval of 7 days after the previous release. Under the same conditions as those described above, 25 healthy and three ToCV-infected tomato plants, which were sprayed with water, were placed in another cage. Virus-free whitefly adults were released into the cage as described above (control of the secondary transmission).

Infection of tomato plants was evaluated 40 days after the first insect release by means of virus detection by RT-PCR, as described above. The results were expressed as infection percentage and analysed statistically. The mean values were compared by the Scott–Knot test at 5% probability.

Temporal and spatial progress of ToSRV and ToCV in screen cages

A total of 25 experimental plots (11 for ToCV and 11 for ToSRV, as well as three healthy controls) were conducted in insect-proof cages (3 × 6 × 2 m) to study the temporal and spatial progress of ToSRV and ToCV infection under controlled conditions. Transplants of the tomato cv. Santa Clara were manually planted *c.* 30 days after sowing in five rows at 0.25 × 0.75 m spacing in a total of 75 plants (15 × 5). The transplants were randomly sampled after transplanting and tested for ToSRV and ToCV infection as described above.

The virus sources of ToSRV and ToCV were tomato plants that were inoculated *c.* 30 days before the acquisition step for both virus isolates. Adults of *B. tabaci* were transferred to polyethylene tubes (50 mL) for a 48 h AAP. The tubes contained one leaflet from a tomato plant infected with either ToSRV or ToCV or one leaflet from a healthy plant (as a negative control); all leaflets were fixed in agar-water. After AAP, two tubes with *c.* 300 whiteflies were placed in the centre of each cage, after which the whiteflies were released. The adult whiteflies were eliminated with an insecticide (thiamethoxam) spray 2 weeks after inoculation. The disease incidence in all plots was evaluated twice per week for symptoms caused by ToSRV and ToCV infection.

Maps representing the 25 plots were generated for each evaluation and used in the temporal and spatial analyses. Plants with typical symptoms of ToSRV and ToCV infection were marked on a 15 × 5 grid at the exact position in each evaluation. A quadrat size of 2 × 2 (two plants in two rows) was used for the spatial analysis. The disease incidence, dispersion index and disease progress curves were determined as previously described.

Results

Spatial and temporal distribution of begomovirus- and crinivirus-infected plants in the field

Visual diagnosis of symptoms

An epidemiological study was carried out during 2012–2014 in 16 plots in four areas of fresh-market tomato production with two hybrid cultivars: Dominador and Predador. Both cultivars are moderately resistant to begomovirus infection, but symptoms were clearly observed on the infected plants. The infected plants showed mild chlorotic spots, mostly on the upper leaves, and symptoms appeared later on other parts of the plant (Fig. 1a,b). Symptoms caused by crinivirus infection appeared later, at least 3 weeks after transplanting; these symptoms consisted of mottling, chlorotic spots, chlorosis and curling, all of which initially occurred in older leaves but subsequently occurred later throughout the plant (Fig. 1c–e). Plants infected with both viruses exhibited typical symptoms of begomovirus infection at the top of the plant and crinivirus infection at the bottom.

Detection and identification of viral species in the field

A total of 317 samples from plants with symptoms were collected from the four fields to confirm the visual diagnosis. All samples were initially analysed by PCR or RT-PCR for detecting begomoviruses or the crinivirus ToCV, respectively. A positive amplification of a DNA fragment of *c.* 1100 bp was obtained in all 177 samples of plants showing symptoms of golden mosaic, confirming that the symptoms were associated with infection. Additional testing of these samples by PCR using ToSRV-specific primers (amplicon of *c.* 800 bp) demonstrated that this virus was present in all samples. The 187 samples from plants exhibiting symptoms of crinivirus infection produced an amplicon of *c.* 500 bp using primers specific to ToCV, confirming ToCV infections in those plants. Both viruses were present in the remaining 47 samples with both sets of symptoms. The PCR products from five randomly selected samples from each field and for both viruses were directly sequenced. A BLAST analysis of the sequences indicated that all 20 samples positive for begomovirus contained DNA corresponding to ToSRV (97–99% nt identity) and that all 20 samples positive for crinivirus contained RNA corresponding to ToCV (99% nt identity).

Temporal analysis

Disease incidence

Symptoms of golden mosaic disease were generally the first to appear in most plots (*c.* 14 days after transplanting

(DAT)), whereas the symptoms of the yellowing disease did not appear until *c.* 21 DAT (Tables S1 & S2).

The incidence of disease and the AUDPC for both diseases varied greatly among the plots, although this variation was low in plots in the same area (Tables S1 & S2). Both diseases had a high average disease incidence and AUDPC in Taquara and Goianápolis, of 67.5% and 216.7, and 29.0% and 104.5, respectively, for golden mosaic disease (Table 1) and 76.0% and 189.0, and 57.8% and 139.8, respectively, for yellowing disease (Table 2). The disease incidence and AUDPC were lower in Anápolis and Boa Esperança, at 6.3% and 19.5, and 1.6% and 10.5, respectively, for golden mosaic disease (Table 1) and 19.8% and 89.3, and 25.3% and 48.2, respectively, for yellowing disease (Table 2). The average disease incidence and AUDPC in the last evaluation ranged from 0.9% to 71% and from 4.2 to 224.2, respectively, for golden mosaic disease and from 4.9% to 87% and from 12.1 to 218.4, respectively, for yellowing disease (Tables S1 & S2).

Disease progress curves

The disease progress curves clearly differentiated between the two virus diseases. For the yellowing disease, although they followed the same pattern of progress as the golden mosaic disease, symptoms developed more slowly, especially in the plots located in Taquara, which is typical for diseases caused by criniviruses (Fig. 2). The incidence of yellowing disease increased later and usually exceeded that of golden mosaic disease. The shapes of the golden mosaic and yellowing disease progress curves were generally similar among all plots, especially among plots at the same location (for example, Taquara in Fig. 2). Evaluations were performed weekly in the first 12 plots evaluated, which were located at Goianápolis, Anápolis and Boa Esperança. However, appropriate adjustments of the curves to any statistical model were not possible, which was probably due to the low frequency of evaluation. The frequency of evaluation was thus doubled at Taquara for the last four plots to two evaluations per week. The disease progress curves for each disease contained fluctuations (waves) within each of the four plots, especially for the yellowing disease. Again, no statistical model could adequately fit any of the curves, indicating that this property is not related to the data collection interval; rather this property is probably inherent to the actual disease dispersion pattern.

Spatial analysis

Ordinary run analysis

The rate of aggregation of plants exhibiting symptoms of golden mosaic and yellowing diseases was first examined by the number of adjacent infected plants by ordinary run analysis. This analysis found that plants with symptoms tended to be randomly distributed. Among totals of 134 (golden mosaic) and 124 (yellowing) maps of plants with symptoms, only 30.6% and 34.7% had an aggregated pattern, respectively (Tables S1 & S2). Among totals of 1249 rows to golden mosaic and 1257 to yellowing, only 56 (4.4%) with golden mosaic and 61 (4.8%) with yellowing showed aggregated patterns of plants with symptoms (Tables 1 & 2). The percentage of plants with symptoms with an aggregated pattern was lower between rows than within the rows. Among totals of 1291 (golden mosaic) and 1276 (yellowing) between-rows evaluations, only 27 (2.1%) with golden mosaic and 23 (1.8%) with yellowing had plants with symptoms distributed in an aggregated pattern (Tables 1 & 2, S1 & S2).

*Dispersion index (*D*)*

A second spatial analysis was based on the dispersion index (*D*). *D* varied among the plots and quadrats for both viruses (Tables 1 & 2, S1 & S2). The *D*-value for golden mosaic and yellowing diseases was usually <1 ($P > 0.05$), indicating a greater tendency for the random distribution of plants with symptoms within the plots. The frequency of $D > 1$ was higher for yellowing (13.7 and 17.7) than for golden mosaic (7.6 and 5.3) in the 2 × 3 and 3 × 2 quadrats, respectively (Tables 1 & 2), suggesting that the distribution of plants infected by ToCV was more aggregated than those infected by ToSRV.

Table 1 Average golden mosaic disease incidence and AUDPC, time of the appearance of first symptoms of the disease, percentages of aggregated plots by *D* analyses, and percentages of aggregated rows and between rows.

			DAT[c]					OR[d]		*D*[e]	
Area	AUDPC[a]	INC[b]	14	21	25	28	35	Rows	B rows	2 × 3	3 × 2
Goianápolis	104.5	29.0	100	100	100	100	100	3.2	1.5	8.7	4.3
Anápolis	19.5	6.3	0	50	50	100	100	2.2	0.0	0.0	9.1
Boa Esperança	10.5	1.6	0	0	0	50	100	0.0	0.0	0.0	16.7
Taquara	216.7	67.5	0	0	25	100	100	5.3	2.6	11.6	1.4
Average	87.8	26.1	25.0	37.5	43.8	87.6	100	4.4	2.1	7.6	5.3

[a]AUDPC, area under disease progress curve.

[b]INC, incidence of golden mosaic disease (%).

[c]DAT, percentages of plots with at least one plant with symptoms at 14, 21, 25, 28 and 35 days after transplanting.

[d]OR, ordinary run, rows (percentage of aggregated rows), and B rows (percentage of aggregation between rows).

[e]*D*, dispersion index, percentages of aggregated plots ($P < 0.05$) in two quadrats (2 × 3 and 3 × 2).

Table 2 Average yellowing disease incidence and AUDPC, time of appearance of the first symptoms of the disease, percentages of aggregated plots by *D* analyses, and percentages of aggregated rows and between rows.

			DAT[c]					OR[d]		*D*[e]	
Area	AUDPC[a]	INC[b]	14	21	28	35	39	Rows	B rows	2 × 3	3 × 2
Goianápolis	139.8	57.8	0	0	75	100	100	5.7	2.57	9.5	4.8
Anápolis	89.3	19.8	0	100	100	100	100	6.6	2.71	25.0	33.3
Boa Esperança	48.2	25.3	0	0	25	100	100	1.5	0	0.0	15.0
Taquara	189.0	76.0	0	0	0	75	100	4.5	1.57	15.3	23.8
Average	116.6	44.7	0.0	25.0	50.0	93.8	100	4.85	1.8	13.7	17.7

[a]AUDPC, area under disease progress curve.
[b]INC, incidence of yellowing disease (%).
[c]DAT, percentages of plots with at least one plant with symptoms at 14, 21, 28, 35 and 39 days after transplanting.
[d]OR, ordinary run, rows (percentage of aggregated rows) and B rows (percentage of aggregation between rows).
[e]*D*, dispersion index, percentages of aggregated plots ($P < 0.05$) in two quadrats (2 × 3 and 3 × 2).

Binary power law

As a third spatial analysis, the binary power law was then applied. The estimated slope and intercept parameters were >1 and >0, respectively, when only the 2 × 3 quadrat was used. These results indicated a slight aggregated pattern of plants with symptoms for both diseases (Fig. 3). For the 2 × 3 quadrats the estimates of b and $\log(A)$ for golden mosaic and yellowing diseases were 1.06 and 0.15 ($R^2 = 0.99$) and 1.05 and 0.14 ($R^2 = 0.94$), respectively, and for the 3 × 2 quadrats were 1.04 and 0.06 ($R^2 = 0.88$), and 1.02 and 0.09 ($R^2 = 0.95$), respectively (Fig. 3).

Dynamic analysis of disease foci (DADF)

To determine whether the golden mosaic disease and yellowing disease progress is predominantly due to primary dissemination, a dynamic analysis of disease foci was performed. The pattern of curves based on the number of clusters was similar for golden mosaic and yellowing, differing primarily only in raw values (Fig. 4a,b). The peak of the total disease cluster was high for both diseases (19 and 21 for golden mosaic and yellowing, respectively). The total disease cluster peaked when the disease incidence of both viruses was similar. The clusters coalesced after the peak in a slow, descending curve. The generalized β distribution function fitted similarly for data for both diseases. The residual values of the plots and the amplitudes of the residual values were 0.90 and 0.95, respectively, for golden mosaic disease and were 18 and 14, respectively, for yellowing disease.

Association between golden mosaic and yellowing diseases

The association between golden mosaic and yellowing diseases was weak in most plots. The rates of association for the plots in Anápolis and Boa Esperança were low and ranged from 0.06 to 0.08 and from 0.00 to 0.04,

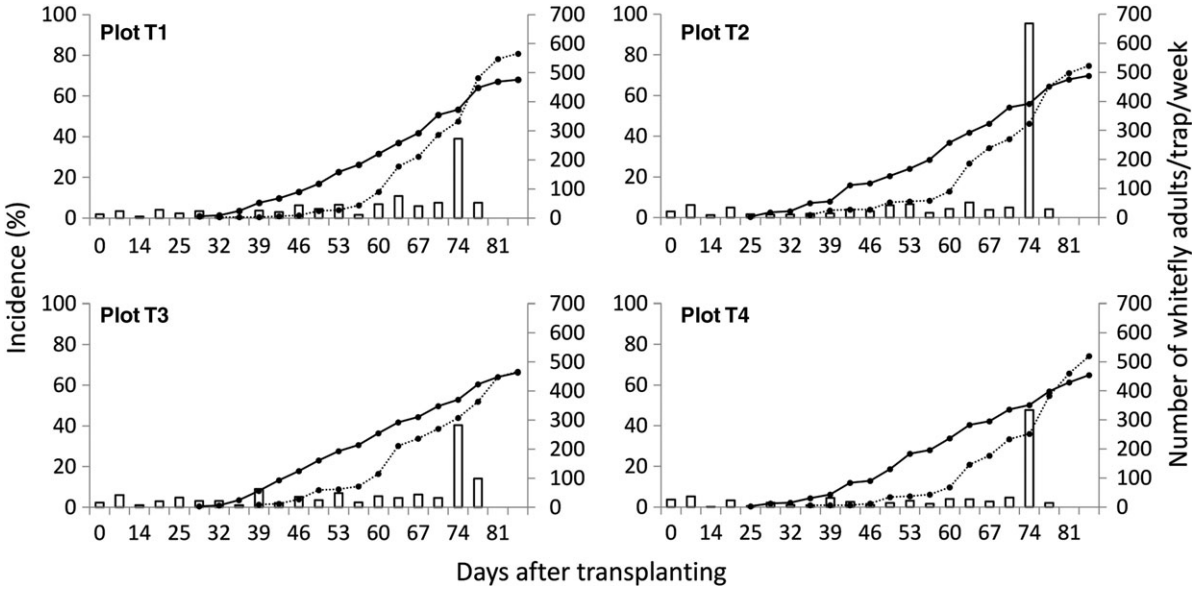

Figure 2 Disease progress curves for golden mosaic disease (solid lines) and yellowing disease (dashed lines) as well as whitefly infestation (bars) in four plots in Taquara.

respectively. The rate of association was intermediate in Goianápolis, ranging from 0.14 to 0.43, and highest in Taquara, ranging from 0.52 to 0.59.

Monitoring whitefly infestation

In tomato fields whose tomatoes are destined for fresh markets, it is normal to spray systemic insecticides (e.g. neonicotinoids and growth regulators) at least twice weekly during the vegetative to flowering stages. The intense insecticide treatments, though, are unable to completely eliminate the whiteflies. Adult insects were observed and trapped on the yellow sticky cards during all evaluations in all plots. The number of trapped *B. tabaci* adults was highly variable. The average number of whitefly adults per plot was highest in Taquara (34.0–58.2 per trap per week), followed by Boa Esperança (12.3–28.8 per trap per week), Anápolis (7.1–15.9 per trap per week) and Goianápolis (8.18–11.0 per trap per week) (Tables S1 & S2). The average number of trapped whiteflies tended to increase throughout the experimental period (e.g., Fig. 2), although the infestation was not uniform among plots. An unusual peak of high infestation of *B. tabaci* was observed in all plots at Taquara at 74 DAT (Fig. 2), possibly due to the arrival of adults from nearby fields. Regular insecticide spraying reduced this infestation, which was observed in the following evaluations. Disease incidence and whitefly infestation were not positively correlated.

Trials under controlled conditions

Transmission of ToSRV and ToCV with B. tabaci *MEAM1*

To evaluate whether a greater incidence of the yellowing disease observed in most fields was due to a difference in the transmission efficiency between ToCV and ToSRV, the transmission rate of ToSRV, ToCV, and both viruses

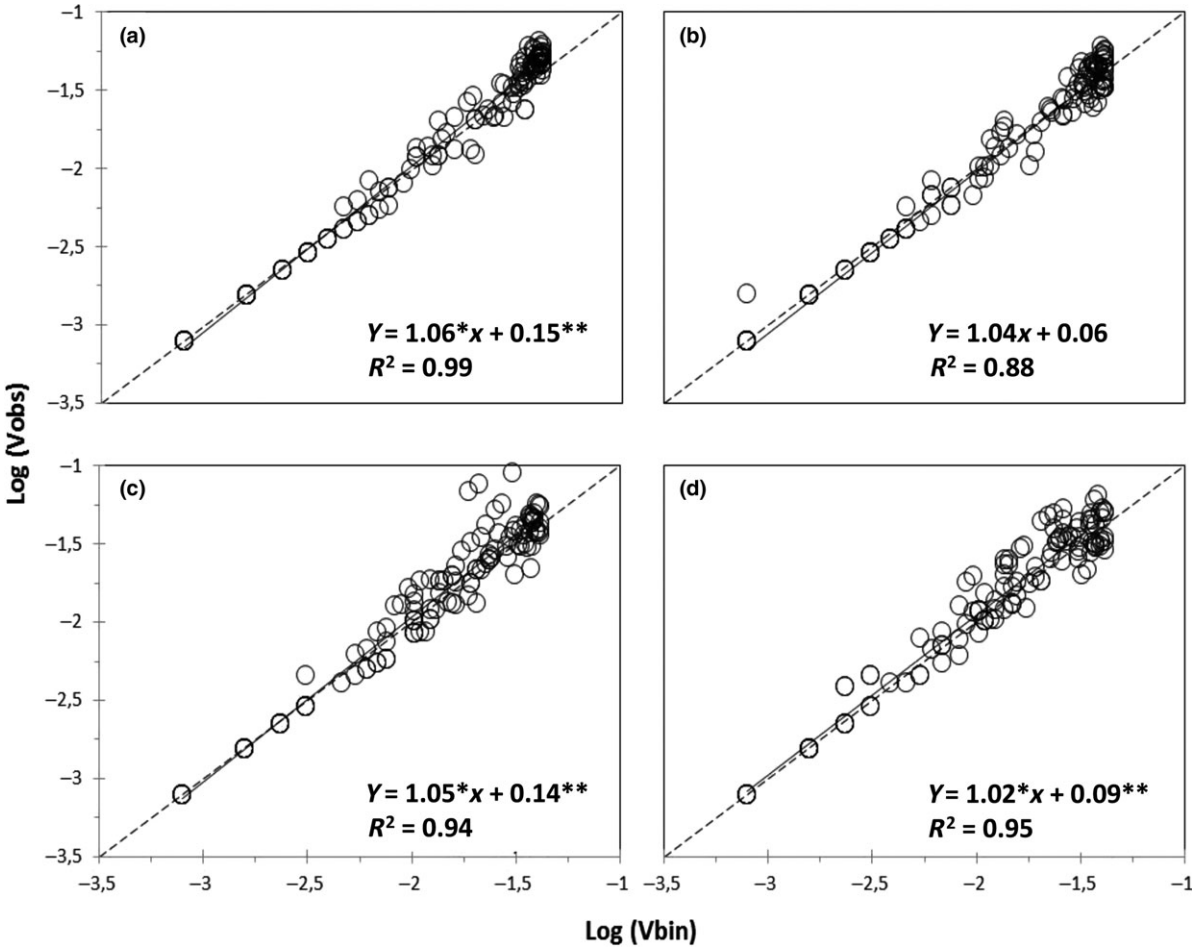

Figure 3 Relationships between the logarithms of the observed variance [log(V_{obs})] and the binomial variance [log(V_{bin})] in all evaluations in all plots for golden mosaic disease [a (2 × 3 quadrat) and b (3 × 2 quadrat)] and for yellowing disease [c (2 × 3 quadrat) and d (3 × 2 quadrat)] in areas of tomato production at Goianápolis, Anápolis, Boa Esperança and Taquara. *Values differ significantly from 1 ($P < 0.05$). **Values differ significantly from 0 ($P < 0.05$). The solid lines represent the fit of the data via the relationship log(V_{obs}) = log(A) + $b$log(V_{bin}) by ordinary least square regression. The dashed lines represent the binomial lines (observed variance = binomial variance).

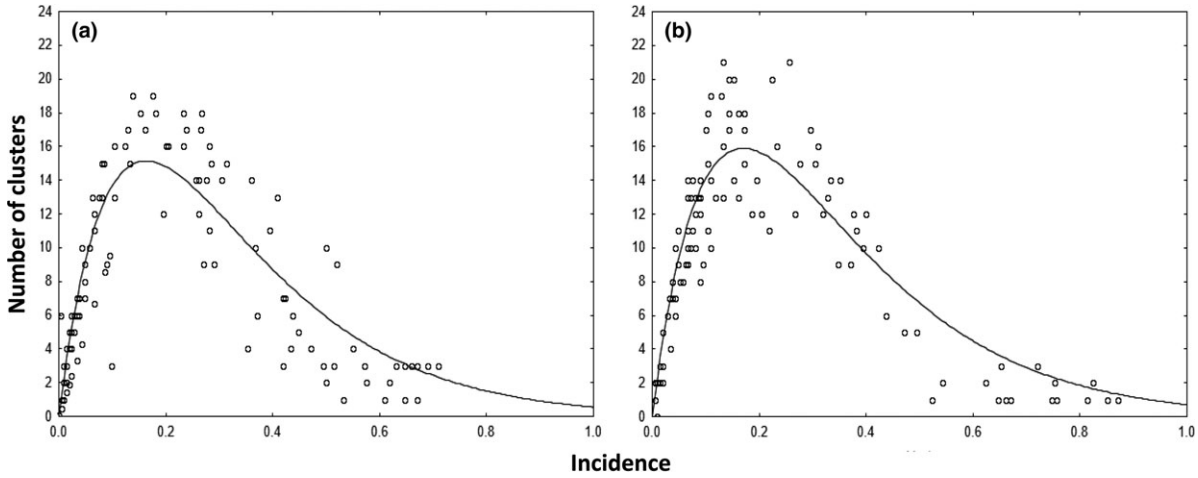

Figure 4 Evolution of golden mosaic (a) and yellowing (b) disease as a function of the incidence and the number of clusters per plot in all plots. The solid lines indicate adjustments of the data to the generalized β function.

was tested using one individual adult insect with an AAP and IAP of 24 h. When the whitefly acquired only ToSRV or ToCV, the respective transmission rates were 47.3% and 44.7%. When both viruses were acquired simultaneously, the transmission rate was 44.7% (Table 3). Uninoculated plants were not infected with any of the viruses, as confirmed by (RT-) PCR. As these viruses showed a similar transmission rate in single and mixed infections, the difference in incidence of both viruses in the field was most probably not caused by differences in transmission efficiency.

Control of primary and secondary transmission with insecticides

It was observed that plants infected with ToSRV and ToCV tended to be randomly distributed in the experimental plots, whereas ToCV-infected plants were slightly more aggregated. A previous study showed that application of the insecticides cyantraniliprole (root drenching and foliar sprays) and cartap (spray) reduced the secondary spread of ToSRV by *c.* 81–94.5% (Gouvêa *et al.*, 2017). It was therefore hypothesized that a reduction in the secondary spread of ToSRV leads to decreased aggregation of infected plants in the field. On the other hand, whiteflies can acquire and transmit ToCV without a latent period, meaning that insecticides may not limit the secondary spread of ToCV. This fact thus could explain a more aggregated pattern of the distribution of ToCV-infected plants in the field compared to that of ToSRV-infected plants. To test these hypotheses, an experiment was conducted to simulate the primary and secondary dissemination of ToCV to tomato plants by whiteflies, which were sprayed with insecticides. The primary transmission rate was slightly higher for most treatments, including the control, than the secondary transmission rate (Table 4). ToCV transmission rates to tomato plants treated with water (control) were 92% and 79% for primary and secondary transmission, respectively. A slight decrease in the transmission rate was observed in tomato plants sprayed with insecticides under simulation of both primary and secondary transmission, albeit without a significant difference. The most effective insecticide was flupyradifurone, compared to the control. This insecticide reduced the primary and secondary transmission rates by 23.9% and 29.1%, respectively. These data confirm that the spraying of insecticides does not control the primary and secondary spread of ToCV and may lead to more aggregated distribution of infected plants in the field.

Temporal and spatial progress of diseases under controlled conditions

To test whether the random distribution of plants infected with ToSRV and ToCV observed in the experimental plots was due to the predominance of primary dissemination, the primary dissemination was simulated and the temporal and spatial progress of both virus diseases under controlled conditions was studied. The incidence of ToSRV and ToCV varied from 7% to 95% and from 12% to 76%, respectively. The disease progress curves of ToSRV and ToCV (Fig. 5) fitted well with the monomolecular model (data not shown). This result may suggest a strong association with the inoculation method, as only one introduction of viruliferous whitefly adults was performed. Mostly, the dispersion index for the two diseases was <1 ($P > 0.05$), indicating a greater tendency of randomness in the distribution of plants with symptoms within the plots. The frequency of $D > 1$ was higher for yellowing disease (19.3% of the evaluation) than for golden mosaic disease (12.5%) using 2 × 2 quadrats, suggesting that the plants infected by ToCV were more aggregated than those infected by ToSRV, which was similar to the observations under field conditions. This result confirms that the tendency toward random distribution of infected plants for both viruses is probably due to the predominance of primary dissemination.

Table 3 Rate of transmission of tomato severe rugose virus (ToSRV), tomato chlorosis virus (ToCV) and both viruses simultaneously, by one adult of *Bemisia tabaci* MEAM1, to tomato plants cv. Kada.

	No. infected plants/no. inoculated plants[a]		
Virus acquired by vector	ToSRV	ToCV	ToSRV + ToCV
ToSRV	18/38	–	–
ToCV	–	17/38	–
ToSRV + ToCV	21/38	21/38	17/38

[a]Results from four independent trials.

Table 4 Rate of transmission of tomato chlorosis virus (ToCV) by *Bemisia tabaci* MEAM1, for tomato plants cv. Kada sprayed with the insecticides cartap, acetamiprid, cyantraniliprole (by soil), flupyradifurone and pymetrozine, simulating the primary (P) and secondary (S) transmissions.

	No. infected plants/ total		
Transmission	Rep. I	Rep. II	Average infection (%)[a]
Control[b]			
P	21/25	25/25	92 a
S	17/24	22/25	79 A
Cartap			
P	21/25	23/25	88 a
S	15/25	21/25	72 A
Acetamiprid			
P	19/24	24/25	87 a
S	18/25	24/25	84 A
Cyantraniliprole (soil)			
P	14/20	17/23	72 a
S	18/25	19/25	74 A
Flupyradifurone			
P	15/22	18/25	70 a
S	11/21	15/25	56 A
Pymetrozine			
P	16/25	22/24	80 a
S	11/24	19/23	64 A

[a]Average values followed by the same letter do not differ from each other by the Tukey test with a significance level of 5%. Lower case and upper case letters compare treatments with primary control and secondary control, respectively.
[b]Tomato plants sprayed with water were used as control.

Discussion

Outbreaks of golden mosaic disease in fresh-market tomatoes are currently common in Brazil, even with the use of cultivars resistant to begomoviruses (Macedo *et al.*, 2014). These cultivars usually carry the *Ty*-1 gene, which is the most widely used gene that confers resistance to tomato yellow leaf curl virus and other begomoviruses (Zamir *et al.*, 1994). Golden mosaic symptoms in resistant tomato cultivars are generally mild, and low levels of losses are expected. The incidence of the yellowing disease, which has been detected in Brazil since 2006, has been increasing over the past decade in the major tomato-producing areas (Barbosa *et al.*, 2008, 2011; Macedo *et al.*, 2014). ToSRV and ToCV are now the two most common whitefly-transmitted viruses found in tomato crops in Brazil (Barbosa *et al.*, 2011; Macedo *et al.*, 2014, 2017).

In the present study, all analysed field plants with viral-like symptoms were infected with ToSRV, ToCV or both, which was confirmed by (RT-)PCR detection. Species-specific primers were used to detect both viruses. As all plants with symptoms were PCR positive, it is unlikely that other begomovirus and crinivirus species occurred in these fields at high incidence. The disease incidence and AUDPC were higher for yellowing than for golden mosaic in all plots. Several factors may have led to this scenario, e.g. the cultivars in the studied areas were moderately resistant to begomovirus, the number of external sources of inoculum was different, or the virus–vector inter-relationships were distinct. The latter hypothesis is unlikely because it was demonstrated that the transmission efficiency of ToSRV and ToCV was similar in both single and mixed infections.

In the experimental plots, the incidence rates for golden mosaic disease and yellowing disease were highest in Taquara, at 71% and 87%, respectively, followed by Goianápolis, with 44% and 82%, respectively (Tables S1 & S2). These two areas share specific characteristics: (i) many growers produce tomatoes throughout the year; (ii) most growers are small producers, with fields of *c.* 5000 plants; and (iii) other vegetables are planted in the surrounding region. Regular infestations of whiteflies throughout the year are therefore common. While Goianápolis and Taquara are traditional tomato-growing regions, those with the lowest viral incidence rates for both diseases, Anápolis and Boa Esperança, are new areas with no history of tomato cultivation. The production areas are small and probably have few alternative hosts that are infected by ToSRV and ToCV.

The disease incidence in Taquara presented similar rates between golden mosaic and yellowing. The plots at this location were very close to a tomato crop in the final stage of production, with high incidence of both diseases (>90%) and with a relatively high whitefly infestation, suggesting that it was the main source of inoculum for the plots in Taquara. In Goianápolis, plots G1 and G2 were located *c.* 0.3 km apart from G3 and G4, and infected plants of plots G1 and G2 might have acted as sources of inoculum for plants in plots G3 and G4, which were transplanted 2 weeks later. Crops that could serve as the main source of inoculum for the plots were not identified in the other areas, because no tomato fields were observed for at least 5 km. This means that non-tomato plants may act as relevant inoculum sources of ToSRV and ToCV, and/or that *B. tabaci* capacity for long-range movement, as discussed by Byrne & Blackmer (1996), may have a significant importance on virus spread. This topic needs further studies.

The association of golden mosaic disease and yellowing disease was low, except in four (Taquara) of the 16

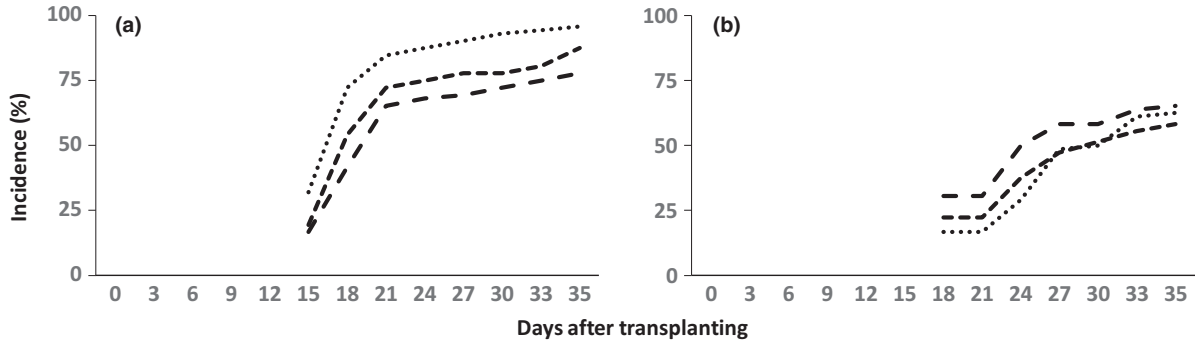

Figure 5 Disease progress curves of golden mosaic (a) and yellowing (b) diseases in three plots under controlled conditions. Different line types indicate different repetitions.

analysed plots, as assessed by the Jaccard index. Similar low associations between viruses transmitted by the same whitefly vector were observed in a cucumber crop between *Cucumber vein yellowing virus*, an ipomovirus species, and *Cucumber yellow stunting disorder virus*, a crinivirus species (Ruiz *et al.*, 2006), as well as in a watermelon crop between a begomovirus (*Cucurbit leaf crumple virus*) and another ipomovirus (*Squash vein yellowing virus*) (Turechek *et al.*, 2014). In the present study, golden mosaic and yellowing diseases were highly associated only in the plots in Taquara, which again supports the premise that the tomato crop in the final stage of production, which was near the experimental plots, was the main source of the viral inocula. Furthermore, under controlled conditions, the transmission efficiencies of ToSRV and ToCV by individual whiteflies that acquired the viruses separately or simultaneously were similar. This result may imply that if uniform ToSRV and ToCV sources are near to young tomato crops, it could be expected that the incidence of both viruses might be similar. Due to the lack of temporal analyses, there is currently no consensus for a model that best fits the disease epidemics caused by a tomato-infecting crinivirus. However, the monomolecular model has been the predominant model for epidemics of the disease caused by begomoviruses in tomatoes (e.g. Polston *et al.*, 1996; Della Vecchia *et al.*, 2007; Barbosa *et al.*, 2016). The golden mosaic and yellowing progress curves in the study here presented similar shapes and were approximately parallel, although the curve for yellowing disease lagged slightly behind the curve for golden mosaic. This difference between the disease curves was most probably due to the longer incubation period of ToCV infection in tomato plants (Tzanetakis *et al.*, 2013). The disease progress curves could not be fitted to established epidemiological models, even for the plots that had a relatively high number of evaluations. These difficulties in fitting an epidemiological model are possibly related to the presence of disease fluctuations in both disease curves.

Fluctuations in the disease progress curves may be due to the influx of viruliferous whiteflies during certain periods in the tomato crops. The disease progress curves of golden mosaic and yellowing obtained from simulations of primary dissemination under controlled conditions fit well to the monomolecular type. This result suggests that after a single influx of viruliferous whiteflies, a monomolecular model fits to the spread of ToSRV and ToCV diseases; thus, the waves observed under the field conditions are consequences of several influxes of viruliferous whiteflies in the target crop. Together these data indicate that ToSRV and ToCV are mainly disseminated by continuous primary spread, confirming what was reported for ToSRV by Barbosa *et al.* (2016) and Macedo *et al.* (2017). Other spatial studies of diseases caused by begomoviruses in tomatoes (Polston *et al.*, 1996; Barbosa *et al.*, 2016) and by crinivirus in watermelons (Turechek *et al.*, 2014) and cucumbers (Ruiz *et al.*, 2006) reported similar findings, strongly suggesting that the infected plants in those fields were randomly distributed.

The dynamic analysis of disease clusters was informative and found many clusters for both diseases, indicating that the plants with symptoms were distributed more randomly in the experimental plots. Similar results were also observed for golden mosaic disease in tomatoes (destined for processing) in plots at the edge of the field, and a good fit of the cluster dynamics was obtained for the generalized β distribution (Macedo *et al.*, 2017). The golden mosaic disease and the yellowing disease dynamics also fit well to a generalized β distribution function. A good fit of the disease foci to the generalized β function indicates that the disease had few modes of dissemination, as was also reported for citrus variegated chlorosis, which has only one mechanism of dissemination (Laranjeira *et al.*, 2004). In contrast, a poor fit indicates that the diseases are spread by more than one major mechanism, as has been reported for citrus canker, which has at least three mechanisms of dissemination (Gottwald *et al.*, 2007). The data suggest that primary spread is the most prevalent mechanism of dissemination for golden mosaic disease and yellowing disease. Although two other minor mechanisms of dissemination were reported for these diseases, these mechanisms are probably less important in this case, as indicated before in studies on processing tomato cultivation (Macedo *et al.*, 2017).

The spatial distribution and temporal progress of both diseases were very similar in this study. This result is surprising, because these viruses are transmitted differently: a persistent transmission for begomovirus, while a semipersistent transmission for crinivirus. The absence of a latent period in the vector for ToCV transmission is expected to produce an aggregated pattern of symptomatic plants, because the vector is immediately able to inoculate a newly acquired virus into healthy neighbouring plants (Wisler & Duffus, 2001). Furthermore, viruliferous whiteflies lose their ability to transmit ToCV after a short period of around 1–3 days (Wintermantel & Wisler, 2006). In contrast, individuals of *B. tabaci* become viruliferous at only about 12–15 h after feeding in ToSRV-infected plants and retain the ability to transmit the virus for longer than the reported 3 days for ToCV (e.g. Toloy *et al.*, 2018). The similarity of the spatial and temporal disseminations of golden mosaic and yellowing diseases may be due to the high frequency of insecticide spraying, which largely eliminates secondary dissemination. Under controlled conditions, it was demonstrated that some insecticide spraying drastically reduces the secondary transmission of ToSRV (Gouvêa *et al.*, 2017) but less intensely reduces (*c.* 20–30% decrease) the secondary transmission of ToCV. This occurrence may therefore result in a slight increase in the aggregation of yellowing disease distribution compared to that of the golden mosaic disease distribution. Furthermore, none of the evaluated insecticides could reduce the primary transmission rates of ToSRV (Gouvêa *et al.*, 2017) or ToCV by whiteflies.

The average whitefly infestation was relatively steady in the plots of the same region, but the number of adults varied widely among the evaluated areas. The number of whiteflies in an area was not positively correlated with the incidence of golden mosaic disease or yellowing disease. Some plots with low whitefly infestation were associated with a high incidence of disease, as noted in the plots in Goianápolis. Variation in the number of whiteflies is unlikely to be the major determinant, as similar results were obtained in studies of begomovirus in tomatoes for processing (Macedo *et al.*, 2017) and in tomatoes destined for fresh markets in Brazil (Barbosa *et al.*, 2016) and Florida (USA) (Polston *et al.*, 1996).

The present study simultaneously evaluated golden mosaic disease and yellowing disease, the two major whitefly-transmitted diseases affecting tomatoes in Brazil, and concluded that the epidemiological dynamics of both diseases were very similar in fresh-market tomato crops. The primary spread of the viruses by the influx of viruliferous whiteflies may be crucial in the development of these epidemics, although three distinct mechanisms can spread these two viruses. Previous work developed by Barbosa *et al.* (2016) and Macedo *et al.* (2017) also found out the importance of primary infection on the epidemics of golden mosaic disease in São Paulo state and Federal District, Brazil, respectively. The key to managing golden mosaic and yellowing diseases may be preventing the arrival of viruliferous insects to production areas. Attempts to control whiteflies only in the target crops with insecticides have been ineffective; it was demonstrated experimentally here that all tested insecticides did not control the primary spread of ToCV, and the same was reported by Gouvêa *et al.* (2017) for ToSRV. Regional and integrated management, not only for tomato crops, but also for crops that can serve as alternative hosts for viruses and/or *B. tabaci* MEAM1, is thus essential as suggested by Bergamin Filho *et al.* (2016).

Acknowledgements

The authors are grateful to the tomato producers in Goianápolis, Anápolis, Boa Esperança, and Taquara for data collection in the tomato fields. The authors thank José Luiz Pereira, Mario Cézar Barbosa, Lucio Flavio Barbosa and Hamilton Jose Lourenço for their technical assistance. This study was supported by grants from Embrapa, the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (proc. no. 482693/2012-6), and Fundação de Amparo à Pesquisa no Estado de São Paulo – FAPESP (proc. no. 2012/51771-4). M.A.M. was the recipient of a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES scholarship, and S.S.B. was the recipient of a CNPq scholarship. A.K.I.N., J.A.M.R. and A.B.F. are CNPq fellows.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Incidence of golden mosaic disease, *D* values (quadrats 2 × 3 and 3 × 2), ordinary run values, whitefly infestation and AUDPC values in 16 plots located at Goianápolis (G1–G4), Anápolis (A1–A4), Boa Esperança (B1–B4) and Taquara (T1–T4).

Table S2. Incidence of yellowing disease, *D* values (quadrats 2 × 3 and 3 × 2), ordinary run values, whitefly infestation, and AUDPC values in 16 plots located at Goianápolis (G1–G4), Anápolis (A1–A4), Boa Esperança (B1–B4) and Taquara (T1–T4).