Spatio-temporal distribution and influence of distance of diseased passion fruit plants in the dissemination of the *Cowpea aphid-borne mosaic virus* in semi-arid tropical region

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Abstract

The *Cowpea aphid-borne mosaic virus* (CABMV), of the genus *Potyvirus* (Potyviridae), causes the most prominent passion fruit (*Passiflora* spp.) disease in Brazil. The virus is transmitted in a non-circulating manner by many species of aphids. Chemical control of the vector insect is inefficient, due to the non-circulating nature of the virus-vector aphid relationship, and commercial species of passion fruit are susceptible to the virus. As such, comprehending the disease's spatio-temporal dissemination dynamic may assist in outlining cultural control strategies, as chemical and genetic control are not used in this situation. The experiments were carried out in commercial fields in the cities of Petrolina-PE and Juazeiro-BA, where the mapping of diseased and healthy plants was carried out over time. The data regarding the incidence of the disease was submitted for analysis with Gompertz, Logistic, Monomolecular and Exponential growth models, in order to determine the best model and the progress rate for the disease's growth curve. The spatial patterns of the disease along the planting lines and over time were determined using the doublets test and through logistic regression, applied to determine the influence of distance between diseased and healthy plants closer to the disease's dissemination pattern. The growth model which best adjusted to the CABMV epidemics in passion fruit plants was the Logistic model, and no difference was observed between the 'r' rates of disease progress. The diseased passion fruit plants exhibited, in both fields, an aggregate pattern along the planting line with similar incidence values (4.16% and 6.74%). The fast development of the disease and the tendency toward aggregation between diseased plants were also confirmed with the analysis of distance through logistic regression. Thus, the production of seedlings in environments that are protected from aphids, systematic inspections for eradication of diseased plants (roguing) and the elimination of host weeds around the orchard may be used as management practices do eliminate inoculum sources and reduce the CABMV's rate of progress.

Keywords: aphids; epidemiology; *Passiflora*; plant disease; virus distribution model and virus transmission.

Introduction

Yellow passion fruit species and reddish and purplish variants (*Passiflora edulis* f. *flavicarpa*) and sweet passion fruit (*P. alata*) comprise the *Passiflora* species with the greatest commercial interest (Martins, et al., 2003; Moreira et al., 2018). Brazil produced around 600 thousand tons of passion fruit in 2019, with an average yield of 14.271 t/ha, and the state of Bahia stands out, accounting for approximately 30% of national production (Lima, et al., 2017; Ibge, 2020). Although the country is the world's greatest producer, the yield per area is considered to be low (Faleiro and Junqueira, 2016).

The passion fruit plant is affected by many pathogens, and the viral infections, especially, stand out due to their fast dissemination when associated with vectors, causing reduction in the plants' vigor and a decrease in productivity and quality of the fruit. The main viral infection which occurs in passion fruit plants is the hardening of the fruits, and the *Cowpea aphid-borne mosaic virus* (CABMV, *Potyvirus* genus, *Potyviridae* family) is the species most frequently associated with the disease in Brazil (Nascimento et al., 2006; Rodrigues et al., 2015). In addition to damaging the fruit, the virus

causes a mosaic, bullous aspect and the deformation of the leaf blade. The virus is transmitted in a non-circulating (non-persistent) manner by many species of aphids, and has caused significant losses and the abandonment of orchards (Rodrigues et al., 2016). According to Cavichioli et al. (2011), the incidence of CABMV in passion fruit orchards can reach 100% in fewer than six months. *Cowpea aphid-borne mosaic virus* (CABMV) isolates in Petrolina-PE were reported, initially, in cowpeas by Batista et al. (2001) through serological tests. Afterwards, studies with passion fruit plants employing techniques for analyzing the amino acid sequence of the capsid protein of isolates from different regions, including Petrolina-PE, indicated that the isolates infecting passion fruit plants were CABMV isolates, rather than *Passion woodiness virus* (PWV, *Potyvirus*), as was previously believed (Nascimento et al., 2004). There is no genetic variability for CABMV resistance in commercial species of *P. edulis* and breeding programs through interspecific hybridizations aimed at developing greater resistance are incipient (Freitas et al., 2015; Preisigke et al., 2020). Chemical control of the vector is unsatisfactory, due

to the virus-vector relationship being non-circulating and to the fact that vector aphid species do not colonize passion fruit plants (Fischer and Rezende, 2016). In the absence of genetic and chemical control, the rational development of cultural control techniques and strategies which prevent the proliferation of the vector and dissemination of the disease must be prioritized.

Studying the speed and manner in which the disease is disseminated in cultivation during an epidemic may contribute to a better understanding of the evolution of the disease, in addition to assisting in the assessment of management tactics. The fact that the species of CABMV vector aphids do not colonize passion fruit plants may suggest a rapid spread of the disease by bites made by the vector while searching for an ideal host (Fischer and Rezende, 2016). On the other hand, it is important to know how diseased passion fruit plants influence the evolution of the epidemic, considering that, since they are not hosts to aphids, quick migration from these vectors to neighboring plants must create a disease dissemination pattern in the field.

If there is a pattern related to distance for the dissemination of the disease, this knowledge could be applied in disease management works, especially in locations with a low history of incidence. Thus, asymptomatic plants can be inspected over time during eradication works, according to the disease's pattern. The quantification of this risk may assist in the eradication of plants, as proposed on by Marcus et al. (1984) for *Citrus tristeza virus* (CTV).

As such, the objective of this study was to quantify the symptom evolution rate in passion fruit plants cause by CABMV, to determine the evolution of the spatial pattern of CABMV within the cultivation area and the importance of distance between healthy and diseased plants in dissemination.

Results

Incidence and diagnosis of passion fruit with CABMV

The incidence of CABMV in the passion fruit fields was high, affecting approximately 2/3 of plants during the studies (Fig.1 and 2). The analyses with indirect-ELISA tests on passion fruit plant leaf samples confirmed the etiology as being CABMV, corroborating reports of occurrences and identification of the pathogen in plant samples from the Petrolina-PE and Juazeiro-BA regions (Nascimento et al., 2004).

Temporal and spatial evolution of CABMV in passion fruit

According to the parameters used in the selection of the best model to describe the disease's growth curves in the studied areas and the absence of tendencies in the distribution of standardized residuals plotted against predicted values, the logistic model was the one that best adjusted to the data regarding incidence of the disease over time (Table 1). The calculation of the confidence interval at 95% probability of the difference between r parameters, referring to the disease's progress rate, in both curves, included the 0 value (-0.014620 to 0.016620). As such, there was no difference between the progress rates of the incidence of the viral infection in both studied areas, demonstrating a similarity in the temporal evolution of the epidemic. The spatio-temporal analyses of CABMV with the logistic model, using distance as an explanatory variable (Table 2), and with the generalized doublets test (Table 3),

allowed us to classify spatial patterns across different points in time. However, due to the low number of diseased plants and the model's questionable adjustment, logistic regression analyses were only adequate from the fourth assessment in Field 1.

In Field 1, the values of the b1 angular coefficient were not significant in the transition between points 3 and 4 and between points 4 and 5, where new occurrences of infections were not dependent on the distance from the closest previously infected plant. However, b1 was significant between points 5 and 6 and between 6 and 7, indicating an influence of distance in the dissemination of CABMV between previously diseased plants and recently diseased plants. Nevertheless, in the transition between points 5 and 6, the relation was positive, with O.R. > 1, indicating a greater probability of incidence of new diseased plants as the distance increased from previously diseased plants, while, between points 6 and 7, the relation was negative (O.R.<1), and the probability of incidence of recently diseased plants decreased as the distance from previously diseased plants increased.

The graph regarding accumulated probability distribution related to distance (Fig. 3) demonstrates the inversion in the transition between points 5 and 6 and points 6 and 7, initially with increasing probability and subsequently with decreasing probability relating to the increase in distance. The interpretation of the model between points 5 and 6 allows us to demonstrate that the odds increased by 61.4% (e^{b1} -1), while, between, point 6 and 7, the odds decreased by 37.4% for each distance unit (1.0 m). Additionally, the doublets test determined random patterns in points 2 and 3, but aggregate patterns in the other points in time (4, 5, 6, 7 and 8), in accordance with the increasing numbers of the standardized values for Z (Table 3).

The results of the logistic regressions referring to Field 2 demonstrate the influence of distance in the spatial distribution of CABMV. The positive and statistically significant value of the b1 coefficient and the O.R., except in the transition between 5 and 6, suggests that the probability of detecting new infected plants increased with distance from previously diseased plants. Although the values of b1 were positive, they decreased in absolute values over each assessment (Table 3), indicating an increase in proximity between diseased plants or compaction between them, as observed in the analyses carried out during the assessments (Fig. 4). This narrowing in the distance between diseased plants is corroborated by the results of the doublets analyses, in which the aggregate CABMV pattern was verified upon the first assessment, displaying increasing standardized values for Z along the points in time.

Discussion

As observed in this and in other works, passion fruit plant cultivation areas may rapidly exhibit incidences close to 100% of infection by CABMV (Cavichioli et al., 2011; Spadotti et al., 2019), and this rapid dissemination of the disease is intimately related to the efficiency of transmission of aphid species by sampling bites (Garcêz et al., 2015), as CABMV is not transmitted via seeds (Narita et al., 2011). According to Garcêz et al. (2015), *Aphis fabae/solanella* and *A. gossypii* are the most important aphid species in the transmission of CABMV. Nonspecific attraction, during migratory flights, by wavelengths in the yellow range (Kennedy et al., 1961;

Table 1. Statistical parameters for nonlinear regressions used to evaluate the most appropriate growth models to describe the progress of *Cowpea aphid-borne mosaic virus* (CABMV) in Petrolina-PE, Field 1 and Juazeiro-BA, Field 2.

	Model	R^2 (%)	MSE (x10^{-4})	r	s(r)
	Gompertz	99.5	7.22	0.049	0.003
Field 1	Logistic	99.2	10.7	0.073	0.006
	Exponential	95.6	58.7	0.035	0.006
	Monomolecular	67.1	421.0	0.008	0.002
	Gompertz	97.8	12.0	0.042	0.004
Field 2	Logistic	98.7	6.73	0.074	0.005
	Exponential	98.6	7.89	0.047	0.004
	Monomolecular	92.81	40.02	0.018	0.003

R^2: coefficient of determination; MSE: Mean squared error; r: slope or growth rate; s(r): standard deviation of estimated r.
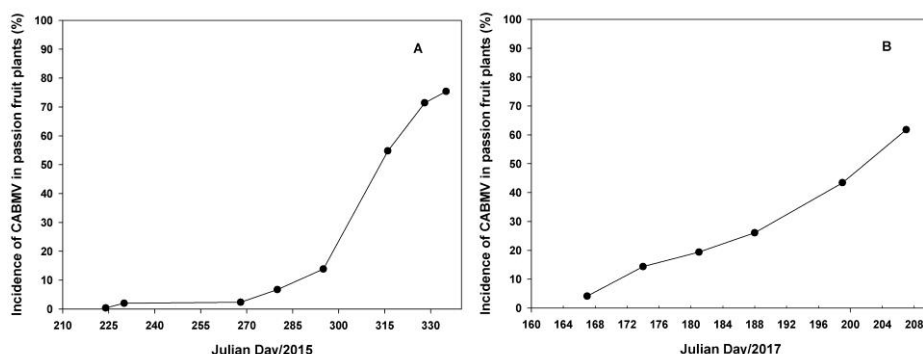

Fig 1. Progress curve for the incidence of *Cowpea aphid-borne mosaic virus* (CABMV) in yellow passion fruit plants in the cities of Petrolina-PE (A) and Juazeiro-BA (B).

Table 2. Results of the logistic regression of the distribution of distance between recently infected passion fruit plants and the nearest plant with CABMV, in different points in time, in orchards in Petrolina-PE (Field 1) and Juazeiro-BA (Field 2). Number of healthy plants, recently infected plants and total diseased plants in absolute values and percentages (%).

	Points in time*	3→ 4	4→ 5	5→ 6	6 → 7	7→ 8
	Healthy plants	235	217	114	72	62
	Recently infected	11	18	103	42	10
	Total diseased	17	35	138	180	190
Field 1	(%)	6.74	13.88	54.76	71.42	75.39
	b_1	1.621	1.437	0.479	-0.468	-14.498
	Pr > chi-square	0.5766	0.1567	<0.0001	0.0063	0.5455
	Odds Ratios (O.R.)	5.063	4.209	1.615	0.626	0.001
	Points in time	**2 → 3**	**3 → 4**	**4 → 5**	**5→ 6**	
	Healthy plants	697	636	478	310	
	Recently infected	46	60	159	168	
	Total diseased	177	238	396	564	
Field 2	(abs./%)	19.40	26.09	43.42	61.84	
	b_1	1.553	0.726	0.308	-0.105	
	Pr > chi-square	0.0048	0.0247	0.0114	0.1767	
	Odds Ratios (O.R.)	4.728	2.067	1.361	0.900	

Field 1: 3-(Julian day 268); 4-(Julian day 280); 5-(Julian day 295); 6-(Julian day 316); 7-(Julian day 328); 8-(Julian day 335). Field 2: 2-(Julian day 174); 3-(Julian day 181); 4-(Julian day 188); 5-(Julian day 199); 6-(Julian day 207).
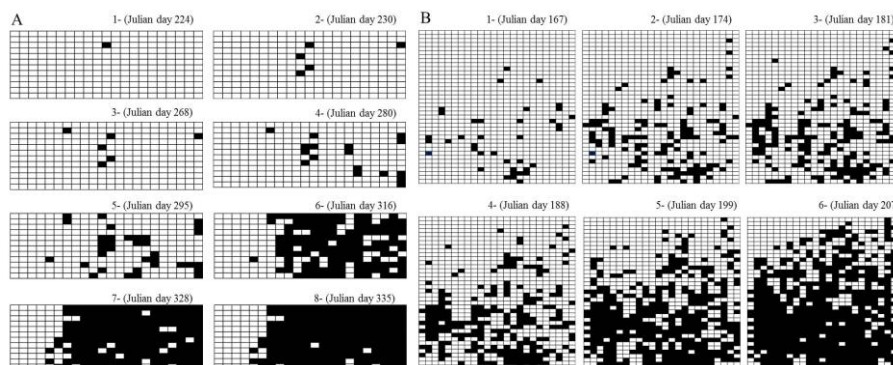

Fig 2. Spatial distribution of passion fruit plants with *Cowpea aphid-borne mosaic virus* (CABMV) along different points in time (Julian day) in Petrolina-PE, Field 1 (A) and Juazeiro-BA, Field 2 (B). Black and white squares represent symptomatic and asymptomatic passion fruit plants, respectively.

Table 3. Spatial distribution of *Cowpea aphid-borne mosaic virus* (CABMV) determined through generalized doublets test proposed by Converse et al. (1979), in commercial passion fruit plant orchards in Petrolina-PE[1] and Juazeiro-BA[2].

Evaluation	N	M	I (%)	r	T	μr	(σr)	Z
1	252	1	0.39	21	0	0.00	0.0000	-
2	252	5	1.98	21	0	0.073	0.26628	-0.2743
3	252	6	2.38	21	0	0.110	0.32491	-0.3372
4	252	17	6.74	21	5	0.993	0.93788	4.2720*
5	252	35	13.88	21	14	4.346	1.82324	5.2950*
6	252	138	54.76	21	96	69.046	4.09750	6.5782*
7	252	180	71.42	21	142	117.669	3.64185	6.6809*
8	252	190	75.39	21	166	131.145	3.41031	10.2204*
Evaluation	N	M	I (%)	r	T	μr	(σr)	Z
1	912	38	4.16	24	8	1.503	1.1773	5.5186*
2	912	131	14.36	24	43	18.202	3.6728	6.7518*
3	912	177	19.40	24	76	33.296	4.6833	9.1183*
4	912	238	26.09	24	121	60.287	5.7950	10.4767*
5	912	396	43.42	24	255	167.183	7.4631	11.7668*
6	912	564	61.84	24	432	339.381	7.3175	12.6571*

N: Total tested plants; M: Cumulative number of infected plants; I (%): Incidence of diseased plants; r: Number of plots; T: Cumulative number of doublets observed; μr: Cumulative number of doublets expected; (σr): Standard error of the expected number of doublets; Z: Standardized value. */Aggregate.
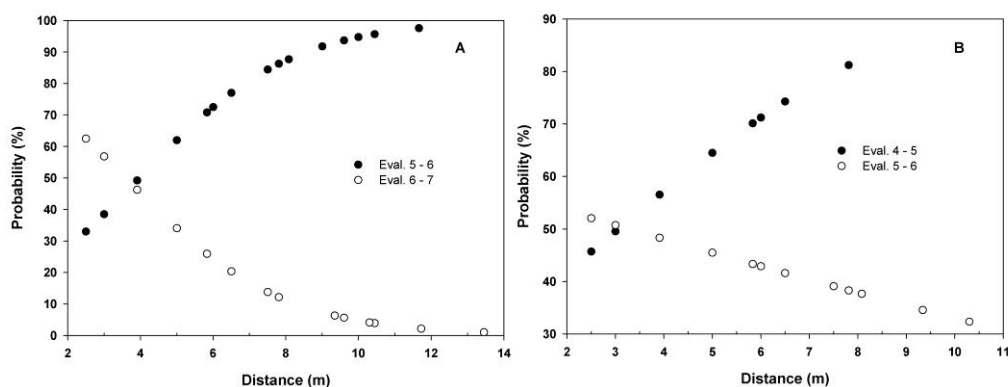

Fig 3. Inversion of the curve from the largest (●) to the smallest (○) probability of incidence of new infected passion fruit plants based on the increase of the distance from the closest previously infected plant, respectively, in the transition between assessments 5-6 (Julian days 295-316) and 6-7 (Julian days 316-328) in Petrolina-PE (A) and in the transition between assessments 4-5 (Julian days 188-199) and 5-6 (Julian days 199-207) in Juazeiro-BA (B).
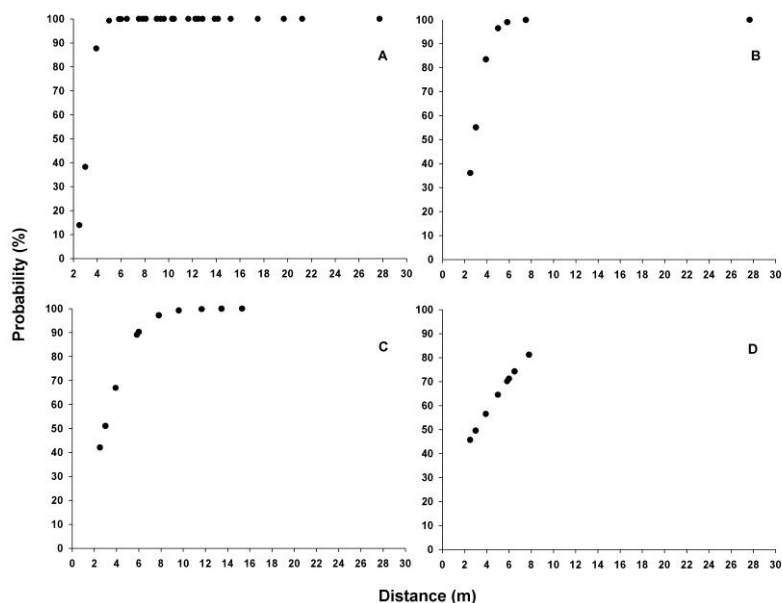

Fig 4. Grouping or reduction in the distance of incidence of new diseased passion fruit plants in relation to the closest previously diseased plants throughout the assessments. A: transition between assessments 2-3 (Julian days 174-181); B: transition between assessments 3-4 (Julian days 181-188); C: transition between assessments 4-5 (Julian days 188-199); and D: transition between assessments 5-6 (Julian days 199-207).

Resende et al., 2007), and the host incompatibility between passion fruit plants and aphid species may partly explain the positive effect of the increase in distance with relation to the incidence of new diseased plants during initial assessments. Additionally, it is expected that vectors in migratory flights from plants contaminated by CABMV and distant from the passion fruit plant fields, as well as transplants of seedlings with latent infections favor an initially random distribution. On the other hand, passion fruit plants and the different species of weeds that are hosts of the virus, located in the borders or within the fields, tend to influence in the contamination of nearby plants (Fischer and Resende, 2016). Knowledge of the characteristics and dissemination pattern of CABMV may assist in cultural management strategies, as there are no products to control the disease, added to the unavailability of resistant commercial cultivars. However, prevention and containment of the spread of the disease may be attained through the production of seedlings in environments that are protected from aphids, as well as control of weeds that host the disease around the cultivation site and the eradication of initial outbreaks within the area, substituting infected plants whenever possible. Indeed, as observed in this work, diseased plants intensified the development of the epidemic, favoring the aggregation of plants with the reduction of dissemination distance over time, as observed by the flattening of the curve in Field 2 (Fig.4), where the model's adjustments proved to be significant. Thus, the subsequent inspection of plants close to the initial outbreaks is important for new eradications, as the probability of new outbreaks arising, especially within 8 meters, is high, as demonstrated.

Systematic employment of the cultural practice of eradication reduced the CABMV growth rate, causing the extension of the passion fruit orchard's useful life (Spadotti et al., 2019). Complete eradication of the orchard at the end of each cycle, establishing a fallowing period along with other producers is equally important (Colariccio et al., 2020). Additionally, we highlight the need for large scale adoption of this practice in order to ensure successful management, similarly to the one practiced for *Papaya ringspot virus* (PRSV-P), *Papaya meleira virus* (PMeV) and *Papaya meleira virus*-2 (PMeV2), in papaya plants in the states of Bahia and Espírito Santo, according to MAPA (Ministry of Agriculture) Normative Instruction Nº 17, dated May 27th 2010, which demands eradication in order to control ringspot and sticky diseases in papaya plants for states that export papaya to the United States (Spadotti et al., 2019; Sá Antunes et al., 2020).

The dearth of a state plan for management favored the quick dissemination of CABMV in passion fruit plant cultivations in the northern region of Santa Catarina, discouraging cultivation and forcing it to migrate to the southern region, which currently faces the same problems **(**Colariccio et al., 2020**)**. The analysis of the spatio-temporal dynamic of the CABMV provided information that can be used as a subsidy for the outline of disease management strategies.

The fast evolution of the diseased observed in both fields compromised the entire orchard's integrity and, presumably, the longevity, quality and productivity of the plants. With the evolution of the diseased, a corrected doublets analysis determined the presence of an aggregate pattern in both fields when incidences were close to each other in 6.74% and 4.16% in field 1 and 2, respectively. This tendency toward aggregation of the disease may be related to the culture's growth pattern forming a barrier and favoring migration of the vector to neighboring plants in planting lines. As such, presumably, planting in a field isolated from other fields, using healthy seedlings and eliminating diseased plants and weeds that host the disease may reduce the risk of a CABMV epidemic through prevention and eradication of the initial inoculum, since the progress of the disease is rapid, as demonstrated. These practices favor management of the disease, enabling a greater productive longevity for the orchard.

Materials and Methods

Location and experimental design
The orchards were installed and monitored in isolated areas from other passion fruit cultivations in the Bebedouro Irrigation Perimeter (PIB) in Petrolina-PE, coordinates (9° 18' 12.3" S, 40 18' 10" W, 364 m), and in the Maniçoba Irrigation Perimeter (PIM) in Juazeiro-BA, coordinates (9° 18' 13.9" S, 40 18' 01.0" W, 374 m), in the years 2015 and 2017, respectively. The area cultivated in the PIB had a size of 1,890 m^2, containing 253 plants in 21 rows with 12 plants each, disposed with a spacing of 2.5 x 3 m, while the area cultivated in the PIM had a size of 6,555 m^2, containing 912 plants, in 24 rows with 38 plants each, disposed with the same spacing.

Assessment of symptoms
In order to determine the temporal and spatial evolution of the CABMV, periodic inspections were carried out to identify symptomatic plants, that is, passion fruit plants that exhibited mosaics, a bullous aspect and deformations in the leaf blade.

Statistical analysis and diagnosis
The disease's spatial dynamics was analyzed through usage of logistic regression, applied to determine the effect of distance between healthy and diseased plants in the dissemination of CABMV, following the methodology of Kleinn et al. (1999). With this analysis methodology, the binary response variable in a specific point in time is the state of the plant, Y, taking on the value Y=1 for recently infected or diseased plants and Y=0 for healthy plants. In this case, the used and tested variable was the distance between a recently infected or healthy plant and the closest already diseased plant. As such, it is considered that the propagation or dissemination of the disease, occurs, in great part, from outbreaks within the cultivation. We also studied the spatial pattern of the viral infection along the planting lines, through the generalized doublets test, following the methodology proposed by Converse et al. (1979). The comparison of the standardized value follows normal distribution, in which, for $Z_D > 1.64$ (P=0.05), the pattern is aggregate and for $Z_D < 1.64$ (P=0.05), the pattern is random,
To quantify the temporal evolution of the disease, the incidence of the viral infection was determined through the relation between the number of diseased plants and the total number plants over time. The progress rate (r) of the viral infection in the passion fruit plants was estimated, and Monomolecular, Logistic, Exponential and Gompertz growth models were adjusted to the data regarding disease incidence over time, through nonlinear regression, through the *PROC NLIN* procedure from the SAS® program. The

adjustments were compared and the best model was selected, based on the highest coefficient of determination between observed and predicted values of incidence, absence of tendency in the residue dispersion graph and the smallest mean square of the residue. The r estimates for each incidence progress curve, in each cultivation area, obtained through one of the aforementioned models, were compared directly, calculating the confidence interval with a 95% probability over the difference between the values of this parameter (Campbell and Madden, 1990).

The confirmation of CABMV in the samples was obtained through serological analyses in enzyme-linked immunosorbent assay tests (indirect-ELISA), using polyclonal antiserum against CABMV and *Cucumber mosaic virus* (CMV) in samples of passion fruit plant leaves.

Conclusions

The growth model, which best adjusted to the CABMV epidemics in passion fruit plants was the Logistic model. Wherein high probabilities of more plants contracting the disease was observed around 8 meters away from previous disease outbreak points, which could be used in eradication works. The results demonstrate the importance of monitoring symptoms in adjacent areas for successful disease management.

Acknowledgments

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