EPIDEMIOLOGICAL ASPECTS OF THE TRANSMISSION AND MANAGEMENT OF COWPEA APHID-BORNE MOSAIC VIRUS IN A PASSION FRUIT ORCHARD

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SUMMARY

Brazil is the world’s main passion fruit (Passiflora edulis Sims) producer. The high incidence of passion fruit woodiness (PFW) induced by Cowpea aphid-borne mosaic virus (CABMV) from the genus Potyvirus, which is transmitted non-persistently by several aphid species, has caused constant and significant production losses, prompting producers to abandon or renew orchards once a year. The present study evaluated the effects of agricultural and ecological conditions on the abundance and composition of aphid populations and the influence thereof on CABMV spread in a passion fruit orchard established with young (30 cm tall) and advanced (80 cm tall) seedlings. The ecological and agricultural characteristics of the region negatively influenced the local aphid population, while aphid swarms and population dynamic were not affected by rainfall, temperature and season. However, when considered together these factors negatively influenced the local aphid population, manifested as low abundance and species diversity, with Aphis gossypii Glover as the most constant and abundant species. Although the virus was introduced simultaneously in both young and advanced seedlings, a longer virus latency period in the latter seedlings resulted in delayed expression of symptoms.

Keywords: Aphids, Aphis, epidemiology, Passifloraceae, Potyvirus, woodiness.

INTRODUCTION

As the country of origin of most passion fruit (Passiflora spp.) species (Bernacci et al., 2014), Brazil is the largest world producer of this fruit with 920,000 metric tons per year grown on approximately 60,000 hectares. Brazil’s northeast and southeast regions account for 94% of the country’s production. Passiflora edulis Sims is the most commonly grown species, representing 95% of overall passion fruit yield (IBGE, 2014; Meletti, 2011). Despite such significant economic importance, passion fruit cultivation is limited by diseases like passion fruit woodiness (PFW) that causes the most significant production losses (Cerqueira-Silva et al., 2014).

In Brazil, PFW is caused exclusively by Cowpea aphid-borne mosaic virus (CABMV) from the genus Potyvirus (Nascimento et al., 2006; Nicolini et al., 2012; Rodrigues et al., 2015). CABMV particles are long, flexuous, and measure on average 750 nm in length. The viral genome consists of a positive sense single-strand RNA with roughly 10,000 nucleotides and two open-reading frames (Barros et al., 2011). Since its first description in Brazil, PFW has been reported in all the country’s passion fruit producing regions, causing a significant decline in both quality and yield. All commercial passion fruit varieties are susceptible to CABMV, which is known to reduce plant size and induce woodiness of fruit, in addition to causing mosaic, blisters, and leaf deformation, resulting in poor pulp yield and low seed numbers per unit (Cerqueira-Silva et al., 2014).

Also, CABMV naturally infects a wide array of commercial plantations as well as natural species of Fabaceae and Passifloraceae (Maciel et al., 2009; Silva et al., 2012). Transmission and spreading of the virus is non-persistently mediated by aphids like Myzus persicae Sulzer, M. nicotianae Blackman, Aphis gossypii Glover, A. fabae Scopoli, A. solanella Theobald, Toxoptera citricidus Kilkaldy, Uroleucon ambrosiae Thomas (Inoue et al., 1995; Gárcêz et al., 2015). Prevention measures include the cultivation of plants kept protected inside structures built with anti-aphid nets. Another option is the establishment of orchards using plants over 80 cm in height, called advanced-stage seedlings (Meletti, 2011).

Understanding the relationships between a heterogeneous environment and the occurrence, distribution, and epidemiology of a phytopathogen is essential as a means to find the most appropriate way to manage crops and to adopt disease control strategies (Nutter, 1997). Therefore, aiming to improve our knowledge of the epidemiology of the pathosystem CABMV-passion fruit, we: (i) surveyed and evaluated the diversity of aphid species; (ii) assessed the influence of season and environmental changes on the occurrence and diversity of aphid species; (iii) identified and predicted the association between aphid species and CABMV dispersal; (iv) evaluated the effect of aphid fauna...
composition on CABMV dispersal; and (v) investigated the incidence and dispersal of CABMV in orchards established with both young and advanced seedlings.

MATERIALS AND METHODS

Selection of the variety of passion fruit, and study site and duration. The orchard investigated was established with Passiflora edulis Sims variety ‘Sul Brasil’ in the Vale do Ribeira Experimental Farm, municipality of Pariquera-Açu, SP (24°36’41.14”S, 47°53’8.07”W, 39 m a.s.l.). Monitoring occurred monthly from October 2012 to November 2013.

Seedlings and establishment of the orchard. The orchard consisted of 200 passion fruit plants including 100 advanced (sown in April 2012) and 100 young (sown in July 2012) seedlings. Only the 40 seedlings growing in the center of each plot were evaluated. The other 60 seedlings were considered border bed. All used seedlings were labeled ‘A’ or ‘B’, for advanced and young seedlings, respectively, and were given a number from 1 to 40. Advanced and young seedlings were allowed to reach 80 cm and 30 cm in height, respectively. All seedlings were kept in an anti-aphid net environment and individually analyzed for the presence of CABMV using the plate trapped antibody – enzyme-linked immunosorbent assay (PTA-ELISA) with CABMV-specific polyclonal antisera before introduction in the orchard in October 2012. The orchard was managed according to the directives established for the cultivation of passion fruit in São Paulo, Brazil (Meletti, 2011). Mean monthly temperature and rainfall were obtained from a weather forecast station in the Vale do Ribeira Experimental Farm.

Leaf samples. Leaf analysis and monitoring of advanced and young seedlings started 30 days after transplantation, and were carried out once a month for 14 months. In the first four months, fragments were collected from leaves above from the third apical node. From the 5th month on, samples were collected at equidistant points, considering the main stem (Garcêz et al., 2015).

Detection and evaluation of CABMV. The time between manifestation of disease symptoms on both advanced and young seedlings (latency period) was estimated based on a visual inspection of leaves and simultaneous collection of leaf samples for PTA-ELISA (Nutter, 1997). Dispersal and incidence rates of CABMV were obtained on a monthly basis based on the results of PTA-ELISA and visual inspection (Garcêz et al., 2015; Gibbs, 1983).

Capture, identification, and determination of peaks in aphid swarms. Aphids were captured using a yellow pan trap built and a plastic tray (W: 35.0 cm, L: 30.0 cm, H: 7.0 cm) filled with water, detergent, and formaldehyde installed 20 cm above the ground (Moericke, 1955). The outside surface of trays was painted brown. Trays were placed on the south and north sides of the orchard. Captured insects were collected periodically and transferred to an appropriate flask, which was then sealed. Aphids were identified using a stereomicroscope and the morphological characters as described by Costa et al. (1993). The dichotomous keys used were specific for aphids (Homoptera: Aphidoidea) (Eastop et al., 1993; Costa et al., 1993; Blackman and Eastop, 2000).

Descriptive analysis of aphids. The abundance and composition of the local aphid fauna were calculated based on the specific diversity index (H’) described by Shannon (1948). The composition of aphid swarms in the orchard was determined using the occurrence and dominance indices proposed by Abreu and Nogueira (1989), whose results, when combined, afford to classify aphids in the orchard as rare (R), intermediate (I), and common (C).

Correlation between environment-aphids-CABMV. The correlation between the number of individuals of a given aphid species in swarms and temperature and rainfall, as well as the influence of these correlation on the dispersal of CABMV, was estimated by simple linear regression (Pearson’s correlation coefficient). Biotic and abiotic parameters were log-transformed using the Log(N + 1) function.

Multivariate analysis of groups and organization of aphid species. The analysis of groups and organization of aphid species was carried out using the Jaccard index obtained according to the unweighted pair group method with arithmetic mean (UPGMA) in the software R version 3.0.2. The variables used to determine the clusters considered the spatial and temporal conditions, that is, the relationships between aphid fauna and environmental conditions.

RESULTS

Detection, incidence, and dispersal of CABMV. The serological analyses revealed that CABMV infection started at essentially the same time in both treatments (young and advanced seedlings). However, the plants grown from advanced seedlings exhibited a comparatively longer latency period (Fig. 1). Also, the detection of CABMV and the emergence of symptoms went from south to north in the orchard, independently of treatment. Whether using PTA-ELISA or observation of symptoms, the first positive diagnoses of CABMV were recorded in plants grown from young seedlings, five months after the establishment of the orchard, which coincided with the transition from
summer to fall. Nevertheless, it should be highlighted that 8.57% of plants were already infected with CABMV and 2.85% presented the typical symptoms induced by the virus. During this period, all plants from advanced seedlings were negative for the virus in PTA-ELISA with no symptoms. Six months after the establishment of the orchard, during fall, 29.41% and 25.64% of the plants grown from young and advanced seedlings, respectively, were positive for CABMV in PTA-ELISA (Fig. 1 and 2). However, plants grown from advanced seedlings had not manifested any symptom by then, while 8.82% of those grown from young seedlings displayed severe leaf symptoms. In the 8th month, at the height of winter, 82.35% of plants grown from young seedlings were positive for CABMV in PTA-ELISA, and 50% of these plants were already showing evident symptoms, though they were comparatively younger in age and therefore smaller in size. Interestingly, it was only during this period that plants grown from advanced seedlings exhibited the first symptoms (5.12% of these plants), though 56.41% were positive for the virus in PTA-ELISA. In the 9th month, 100% of plants grown from young seedlings exhibited serious symptoms of CABMV infection. Nevertheless, 17.5% of plants grown from advanced seedlings were asymptomatic. It was only in the 14th month that all plants grown from advanced seedlings (100%) showed symptoms of infection with CABMV (Fig. 1 and 2).

However, the Shannon index ($H_1 = 0.977$) showed that the local aphid population was characterized by low species diversity. The correlation between mean temperature and rainfall recorded in the Vale do Ribeira region during the survey period indicated that these factors did not influence aphid swarms. Mean temperature in the area during the study was 21.2°C, and had a barely weak negative relationship with swarms ($R = -0.3145$). In turn, mean rainfall was 119.4 mm, with a barely positive relationship with aphid swarms ($R = 0.0498$) (Tables 1 and 2). Also, the most significant aphid swarms were observed in fall (when mean temperature varied between 15°C and 25°C and total rainfall reached 221 mm) and in winter (mean temperature in the 12°C to 21°C interval and total rainfall of 181 mm) (Table 1).

Concerning abiotic factors, half of the aphid species captured were polyphagous ($A. craccivora$, $A. fabae$, $A. gossypii$, Table 1. Influence of abiotic (temperature and rainfall) and biotic (height of host plant and abundance and diversity of aphid species) factors on epidemiology of Cowpea aphid-borne mosaic virus (CABMV) in an orchard of Passiflora edulis Sims variety 'Sul Brasil' in the municipality of Pariquera-Açu (Vale do Ribeira, SP, Brazil).

<table>
<thead>
<tr>
<th>Months/Year</th>
<th>Temp (°C)¹</th>
<th>Rainfall (mm)²</th>
<th>Young seedlings</th>
<th>Advanced seedlings</th>
<th>Status</th>
<th>Aphids species</th>
</tr>
</thead>
<tbody>
<tr>
<td>November/2012</td>
<td>21.0</td>
<td>92.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>December</td>
<td>331.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>January/2013</td>
<td>22.2</td>
<td>264.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>February</td>
<td>25.9</td>
<td>126.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>March</td>
<td>23.8</td>
<td>219.6</td>
<td>8.5</td>
<td>2.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>April</td>
<td>21.8</td>
<td>35.9</td>
<td>29.4</td>
<td>8.8</td>
<td>25.6</td>
<td>-</td>
</tr>
<tr>
<td>May</td>
<td>20.0</td>
<td>55.7</td>
<td>64.7</td>
<td>14.7</td>
<td>48.7</td>
<td>-</td>
</tr>
<tr>
<td>June</td>
<td>18.2</td>
<td>121.2</td>
<td>82.3</td>
<td>55.8</td>
<td>56.4</td>
<td>5.1</td>
</tr>
<tr>
<td>July</td>
<td>16.3</td>
<td>83.8</td>
<td>100</td>
<td>97.0</td>
<td>66.6</td>
<td>17.9</td>
</tr>
<tr>
<td>August</td>
<td>17.0</td>
<td>22.3</td>
<td>100</td>
<td>97.0</td>
<td>69.2</td>
<td>30.7</td>
</tr>
<tr>
<td>September</td>
<td>16.3</td>
<td>90.7</td>
<td>100</td>
<td>100</td>
<td>79.9</td>
<td>46.1</td>
</tr>
<tr>
<td>October</td>
<td>20.8</td>
<td>43.4</td>
<td>100</td>
<td>100</td>
<td>92.3</td>
<td>69.2</td>
</tr>
<tr>
<td>November</td>
<td>22.7</td>
<td>129.9</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>82.5</td>
</tr>
<tr>
<td>December</td>
<td>27.0</td>
<td>55.5</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Annual mean | 21.2 | 119.4 | - | - | - | - | - | 672 |

General classification of the aphid species recorded: (C) common, (I) intermediate, (R) rare; 1 = monthly mean temperature; 2 = Total monthly rainfall; 3 = Aphid abundance estimate (m²).
Table 2. General classification of the aphid species recorded in the 14-month-long survey carried out in an orchard of Passiflora edulis Sims variety ‘Sul Brasil’ in the municipality of Pariquera-Açu (Vale do Ribeira, SP, Brazil, 2012-2013), considering rainfall and temperature.

<table>
<thead>
<tr>
<th>Species</th>
<th>Classification (14 months)</th>
<th>Rainfall ‘r’ (Correlation)</th>
<th>Temperature ‘r’ (Correlation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphid craccivora</td>
<td>I</td>
<td>0.177 (Bn)</td>
<td>-0.348 (Wn)</td>
</tr>
<tr>
<td>A. fabae</td>
<td>I</td>
<td>-0.087 (Bn)</td>
<td>-0.698 (Mn)</td>
</tr>
<tr>
<td>A. gossypii</td>
<td>C</td>
<td>0.203 (Wp)</td>
<td>0.039 (Bp)</td>
</tr>
<tr>
<td>A. nasturtii</td>
<td>C</td>
<td>-0.013 (Bn)</td>
<td>-0.447 (Wn)</td>
</tr>
<tr>
<td>A. nerii</td>
<td>I</td>
<td>0.109 (Wp)</td>
<td>0.213 (Wp)</td>
</tr>
<tr>
<td>A. spiraeola</td>
<td>I</td>
<td>-0.313 (Wn)</td>
<td>-0.548 (Mn)</td>
</tr>
<tr>
<td>Aulacorthum solani</td>
<td>I</td>
<td>-0.621 (Mn)</td>
<td>-0.374 (Wn)</td>
</tr>
<tr>
<td>Brevicoryne brassicae</td>
<td>R</td>
<td>-0.081 (Bn)</td>
<td>0.202 (Wp)</td>
</tr>
<tr>
<td>Hyperomyzus lactucae</td>
<td>R</td>
<td>-0.166 (Wn)</td>
<td>0.532 (Mn)</td>
</tr>
<tr>
<td>Macrosiphum euphorbiae</td>
<td>R</td>
<td>0.345 (Wp)</td>
<td>-0.077 (Bn)</td>
</tr>
<tr>
<td>Myzus persicae</td>
<td>I</td>
<td>0.508 (Mp)</td>
<td>-0.121 (Wp)</td>
</tr>
<tr>
<td>Pemphigus spp.</td>
<td>C</td>
<td>-0.131 (Wn)</td>
<td>-0.260 (Wn)</td>
</tr>
<tr>
<td>Pentalonia nigronervosa</td>
<td>R</td>
<td>-0.374 (Wn)</td>
<td>0.126 (Wp)</td>
</tr>
<tr>
<td>Toxoptera aurantii</td>
<td>I</td>
<td>0.093 (Bp)</td>
<td>0.060 (Bp)</td>
</tr>
<tr>
<td>T. citricidus</td>
<td>R</td>
<td>-0.113 (Wn)</td>
<td>-0.414 (Wn)</td>
</tr>
<tr>
<td>Undeleeuw ambrosiae</td>
<td>R</td>
<td>-0.105 (Wn)</td>
<td>0.273 (Wp)</td>
</tr>
</tbody>
</table>

Classification of aphid species: Common (C), Intermediate (I), Rare (R).

‘R’ correlations observed: Weak negative (Wn), Weak positive (Wp), barely negative (Bn), barely positive (Bp), Moderate negative (Mn), Moderate positive (Mp).

The Aphis genus was the most abundant and diverse, with annual population estimated at 5,076 individuals m\(^{-2}\), which corresponds to 85.15% of the total local aphid population. Taking into account that aphids were collected in four seasons defined based on climatic periods, it was possible to observe that A. gossypii was the most abundant species in all seasons, accounting for 53% of the Aphis genus captures. However, it was in fall that this species formed the largest swarm (1,933 individuals m\(^{-2}\)), which corresponded to 32.42% of the total number of aphids captured. However, it was in spring that the highest aphid diversity was recorded, with 15 species, while the lowest abundance (914 individuals m\(^{-2}\)) and diversity (seven species) were observed in summer (Table 1).

The spatial-temporal similarity analyses carried out using the Jaccard index, with a cutoff value of \( \alpha = 0.76 \), revealed that the local aphid population was characterized by two distinct clusters, independently of environmental conditions (Fig. 3). One cluster (G1) was formed by 10 species, representing 62.5% of the total number of species captured. Descriptive analyses also showed that 80% of the G1 species were polyphagous and classified as common or intermediate (A. craccivora, A. spiraeola, A. solani, M. persicae, and T. auranti) and 50% were oligophagous (A. nerii, B. brassicae, H. lactucae, Pemphigus spp., P. nigronervosa, T. citricidus, and U. ambrosiae). According to the general classification index of the aphid population composition, A. gossypii, A. nasturtii, and Pemphigus spp. were common species, accounting for 72.5% of the population. The species A. craccivora, A. fabae, A. nerii, A. spiraeola, A. solani, M. persicae, and T. auranti were classified as intermediate (representing 22.5% of the local aphid population), while B. brassicae, H. lactucae, M. euphorbiae, P. nigronervosa, T. citricidus, and U. ambrosiae were rare species (4.95% of the population).

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**DISCUSSION**

The descriptive and temporal analyses revealed that the aphid population in the passion fruit orchard investigated...
had low species diversity, though high abundance values (Table 1). Therefore, it was possible to conclude that abundance was the main factor to explain the introduction of CABMV in the orchard (as primary infection) and the spread of the virus (secondary infection). These aphid species were mostly polyphagous, and were classified either as common or intermediate (Table 2). Other studies also found out that, of the 16 aphid species captured in the present investigation, A. craccivora, A. fabae, A. gossypii, M. persicae, T. citricidus, and U. ambrosiae had been described as CABMV vectors in the country (Inoue et al., 1995; Garcéz et al., 2105). Similar results were reported in a previous study carried out in the east of São Paulo, Brazil where, despite the recent introduction of passion fruit orchards, 14 aphid species were recorded. Among these, the most common were A. gossypii and A. solanella (which had CABMV transmission efficiency of 40%) (Garcéz et al., 2015). Differently, in a passion fruit orchard in Kenya, 12 aphid species were described for which A. gossypii, A. fabae, Ropalosiphum maidis Fitch, B. brassicae, and Sectobium avenae Fabricius were the most efficient at transmitting CABMV (Kilalo et al., 2013).

The aphid population profile described in the present study was directly correlated with the characteristics of the Vale do Ribeira ecosystem, where areas exposed to low anthropogenic influence occur side by side with vast forest fragments. This double-feature physiognomy induces the emergence of a poorly diverse arthropod fauna (Zimmerman and Kormos, 2012). The aphid swarm profile observed was probably influenced by inherent characteristics of the Atlantic Forest, not rainfall or temperature. It is known that most aphid species are native to temperate environments. This explains why these organisms find it difficult to locate and colonize host plants in their attempts to establish populations in tropical and subtropical regions. The considerable floristic diversity of such environments prompts aphids to perform several recognition stops during flights (Stern et al., 1995). This scenario is quite common in Vale do Ribeira, since 60% of the region is dedicated to conservation units, which incidentally form the largest continuous Atlantic Forest area in the biome (Martini et al., 2007). In addition, this biome is one of the 34 hotspots for plant biodiversity in the planet, and its preservation is enforced by law. Accordingly, the large forested areas nearby production belts influence the agricultural characteristics of plantations (Mittermeier et al., 2004).

Although regression analysis showed a very weak negative correlation between the aphid/temperature indices, daily means did not exceed 22°C. In other words, these mean temperature values may have driven the establishment of aphid populations. This has also been reported by Charnov and Gillooly (2003), who found that temperatures between 10°C to 25°C do not affect the biology of most of the aphid species native to temperate climates that have adapted to tropical and subtropical conditions. Temperatures above 25°C are known to inhibit the biological activities of winged aphid forms, directly interfering in the mobility of these arthropods. The mean temperature in the summer was 25.9°C, which affected the flying behavior of aphids, ultimately explaining the very weak negative correlation observed. This may have had some kind of negative epidemiological impact on the dispersal of CABMV, interfering in the mobility of aphids (Katis et al., 2007).

The climatic conditions in the surveyed area favored the development of colonies and swarms of A. gossypii, since temperatures between 10°C to 25°C are ideal for this species (Soglia et al., 2002). Under these conditions, another trait that boosts the predominance of A. gossypii is this species’ marked polyphagia. For example, a previous study showed that the species interacts with as many as 910 plant species of 116 families (Blackman and Eastop, 2011). Such high species diversities above 25ºC are known to inhibit the biological activities of winged aphid forms, directly interfering in the mobility of these arthropods. The mean temperature in the summer was 25.9°C, which affected the flying behavior of aphids, ultimately explaining the very weak negative correlation observed. This may have had some kind of negative epidemiological impact on the dispersal of CABMV, interfering in the mobility of aphids (Katis et al., 2007).

The climatic conditions in the surveyed area favored the development of colonies and swarms of A. gossypii, since temperatures between 10°C to 25°C are ideal for this species (Soglia et al., 2002). Under these conditions, another trait that boosts the predominance of A. gossypii is this species’ marked polyphagia. For example, a previous study showed that the species interacts with as many as 910 plant species of 116 families (Blackman and Eastop, 2011). Such striking polyphagia places A. gossypii as the aphid species that best adapted to tropical and subtropical conditions. Also, the species has been reported to be the main CABMV vector in passion fruit orchards, with transmission rates of up to 71% (Kilalo et al., 2013). However, according to the analysis of the spatial-temporal composition of
aphid species in the orchard surveyed in the present study, CABMV dispersal was not a one-off event that depended on the predominance of *A. gossypii*; rather, the virus is thought to have spread due to the interaction of *A. gossypii* with five other *Aphis* species that formed cluster G1. Interestingly, this was also observed in the west of São Paulo, in a study that concluded that, although CABMV may also be spread by several aphid genera, the *Aphid* genus was the most abundant (Yuki et al., 2006). The same population profile was observed in the east of São Paulo, where 55.42% of the species identified belonged to the *Aphis* genus. *Aphis solanella* and *A. gossypii* were the most abundant, and were classified as common and intermediate, respectively (Garcêz et al., 2015).

Although *A. fabae* is an intermediate species that is not quite abundant, its proved efficiency in CABMV transmission afford to conclude that it is the main species in the epidemiology of PFW (Kilalo et al., 2013; Garcêz et al., 2015). Also, the fact that cluster G2 did not considerably influence the transmission and spreading of CABMV may be explained in light of the fact that it was formed by a smaller number of exclusively oligophagous species. A similar population dynamics was observed in a passion fruit orchard in the east of São Paulo, in what seems to be a typical feature of aphid populations in subtropical regions (Garcêz et al., 2015). One of the species that clustered in G2, *B. brassicae*, played an important role in the epidemiology of CABMV, since it has been shown to transmit CABMV with an efficiency rate of up to 25% (Kilalo et al., 2013). Here, the oligophagous habit of *B. brassicae* may have helped spread the virus, since this species is known for carrying out inordinate migration flights over crop areas. Therefore, in light of the reports of constant migration flights and the effective role they play in CABMV transmission, it was possible to infer that, in the absence of polyphagous species in G1, *B. brassicae*, despite being an oligophagous species, did play a relevant role in the dispersal of the virus in the passion fruit orchard surveyed. According to Sousa-Silva and Ilharco (1995), Brazilian passion fruit plants are not colonized by any of the species clustered in G1 and G2. Considering the irregular behavior of the aphid species and the low indices of diversity, swarms, and population density, it was concluded that agricultural and ecological characteristics directly influenced the local aphid population. This may be explained considering the fact that fragments of plantations and forests tend to have negative effects on organisms that inhabit such areas, changing original richness and abundance scenarios and even leading to the elimination of some species while others find the appropriate conditions to establish their own populations in these regions (Didham et al., 1996). This became evident in the local aphid population profile, since most of the vector aphid species are associated with open fields exposed to high anthropogenic influence (Hooks and Fereres, 2006).

Independently of growth stage (young or advanced), the passion fruit seedlings used to establish the orchard surveyed in the present study were equally susceptible to CABMV, though advanced seedlings expressed symptoms of infection only later on (Fig. 2). The introduction and fast spread of CABMV was mediated by the local aphid population, characterized by high abundance of common and intermediate species with polyphagous or oligophagous habits. Passion fruit plants are not colonized by any of the 116 aphid species described in Brazil (Souza-Silva and Ilharco, 1995), which suggests that the aphid species reported in the present study were temporary vectors of CABMV. This means that, though they were living in the area, they did not colonize passion fruit plants. This condition was also observed in passion fruit orchards surveyed in Côte d’Ivoire, where *A. spiraeccola* was reported to be the main vector of another potyvirus, *Passionfruit ringspot virus* (Wijs, 1974).

In Brazil, transmission experiments showed that 15 wild Passifloraceae species were susceptible to different CABMV isolates (Maciel et al., 2009). Of the species challenged with the virus, *Passiflora alata* (Dryand), *P. amethystina* (J.C. Mikan), and *P. setacea* (DC) are native to the Atlantic Forest. In addition, several species of Fabaceae have been reported to host CABMV (Nicolini et al., 2012; Silva et al., 2012). Therefore, these plant species may be potential reservoirs of CABMV, with a prominent role in this virus’ epidemiology. So, Passifloraceae and Fabaceae species native from the Atlantic Forest near or even next to areas where passion fruit culture follows intensive agriculture practices very likely play a role in the maintenance of CABMV inoculum sources (Silva et al., 2012).

The CABMV isolates obtained from passion fruit plants in different producing regions in São Paulo, Brazil (including Vale do Ribeira) are phylogenetically related, and tend to form a consistent monophyletic group (Rodrigues et al., 2015). Despite the relatively low genetic variability between the Brazilian CABMV isolates and notwithstanding the large diversity of native passion fruit species described in the country, efforts to develop resistant varieties with commercial value have not been met with any success. However, in recent years much research has been devoted to obtain CABMV-resistant varieties based on crossings between *P. edulis* and *P. setacea* (Freitas et al., 2015; Santos et al., 2015). Consequently, while resistant varieties are not available, the use virus-free advanced seedlings has been one of the methods of choice to produce passion fruit in São Paulo, Brazil. In the present study, in spite of the fact that both young and advanced passion fruit seedlings were equally susceptible to CABMV, it is important to highlight that symptoms of viral infection emerged only late into the study period. This underscores the importance of this technique to passion fruit farmers in Vale do Ribeira, SP, Brazil, who still resort to young seedlings to establish their orchards. In addition to reducing production and establishment costs, the use of advanced seedlings as described in the present study may also lend greater longevity to orchards. Until the 1990s, before the onset of the CABMV...
epidemics, these orchards were maintained productive for at least three consecutive years (Sampaio et al., 2008).

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