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Long-lasting systematic roguing for effective management of CABMV in passion flower orchards through maintenance of separated plants

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In Brazil, passion flower is grown across almost the entire country. The predominant disease of the passion flower crop is passion fruit woodiness, caused by the potyvirus cowpea aphid-borne mosaic virus (CABMV), and transmitted by aphids in a nonpersistent manner. The disease reduces the useful life of the orchard from 36 months to approximately 18 months. Up to now, there has not been an efficient method for disease management. The aim of this work was to evaluate the efficiency of systematic roguing of diseased plants through weekly inspections, for disease management in the field. The latent and incubation periods of CABMV in passion flower vines were determined in order to optimize roguing efficiency. Passion fruit plants inoculated with CABMV started to act as sources of inoculum from 3 days after inoculation (DAI), and the symptoms were expressed, on average, at 8 DAI. Five field experiments, conducted in the states of São Paulo and Bahia, Brazil, demonstrated that systematic roguing of diseased plants was significantly efficient for managing passion fruit woodiness disease. In order to facilitate identification and subsequent removal of the infected plants, they need to be grown separately. This cultural practice can be recommended for managing passion fruit woodiness disease, provided it is applied on a regional scale by all passion fruit growers. The development of some pilot plantings for the application of roguing in a passion flower-producing region is recommended to validate the use of this technique for managing passion fruit woodiness disease.

Keywords: aphid transmission, *Passiflora*, potyvirus

Introduction

Passion flowers are widely grown tropical plants belonging to the family Passifloraceae and the genus *Passiflora*. Of more than 150 species of *Passiflora*, the yellow passion fruit (*Passiflora edulis*) comprises almost the entire volume traded worldwide (Bernacci *et al.*, 2008; Coelho *et al.*, 2016). The most cultivated species in Brazil are the yellow passion fruit and the sweet passion fruit (*Passiflora alata*). Brazil is the world's largest producer of passion fruit, producing 703 489 tonnes in a cultivated area of 49 889 ha in 2016, with a mean yield of 14.1 tonnes ha^{-1} (IBGE, 2017).

The intensification of passion flower cultivation in Brazil has been accompanied by an increase in the incidence of various diseases, especially passion fruit woodiness caused by cowpea aphid-borne mosaic virus (CABMV; Fischer & Rezende, 2008; Garcêz *et al.*, 2015). CABMV (family *Potyviridae*, genus *Potyvirus*, species *Cowpea aphid-borne mosaic virus*) has elongated and flexuous particles measuring 680–900 nm in length and 11–20 nm in diameter. The genome consists of a single-stranded, positive-sense RNA molecule (Wylie *et al.*, 2017).

The symptoms induced by CABMV in passion flower include leaf mosaic, blisters and deformation, as well as some fruit woodiness and reduced size of fruits, making them unmarketable (Nascimento *et al.*, 2006). CABMV also reduces the useful life of the orchards from 36 months to approximately 18 months (Fischer & Rezende, 2008; Correa *et al.*, 2015). The virus is spread mainly by aphids in a nonpersistent manner. *Aphis craccivora*, *A. fabae*, *A. gossypii*, *Myzus nicotianae*, *M. persicae*, *Toxoptera citricidus* and *Uroleucon ambrosiae* have been reported as vectors of CABMV (Costa *et al.*, 1995; Inoue *et al.*, 1995). The virus can be transmitted mechanically by shears and fingernails during

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cultural pruning and trimming practices. Seed transmission does not occur (Fischer & Rezende, 2008).

Control of passion fruit woodiness disease is difficult, mainly because commercially exploited *Passiflora* species are not virus-resistant or disease-tolerant. In addition, chemical control of the insect vector is not efficient, as the virus is transmitted in a nonpersistent manner and the species of aphid vectors do not colonize passion flower (Fischer & Rezende, 2008).

Several disease-control strategies have been evaluated but have not achieved the desired results. These include the use of mild strains of the virus for pre-immunization (Novaes & Rezende, 2003), the identification of genotypes of *Passiflora* species that are resistant or tolerant to CABMV (Maciel *et al.*, 2009; Santos *et al.*, 2015; Gonçalves *et al.*, 2018), and the use of resistance derived against the pathogen through transgenic passion flowers with the CABMV capsid protein gene (Alfenas *et al.*, 2005; Monteiro-Hara *et al.*, 2011; Correa *et al.*, 2015).

Management alternatives that are often recommended to reduce the damage caused by CABMV in passion flower include the use of healthy seedlings produced in certified nurseries, the use of seedlings of at least 100–120 cm in height to establish new orchards, the elimination of old and/or infected passion fruit plants before a new planting, disinfection of pruning tools to prevent mechanical transmission of the virus and planting in isolated locations away from known virus sources of inoculum, whenever possible (Fischer & Rezende, 2008; Cerqueira-Silva *et al.*, 2014; Garcêz *et al.*, 2015). However, these recommendations are not completely effective, and even when they are partially applied, passion flower orchards are renewed annually because of the high rates of infection of plants with CABMV (Cerqueira-Silva *et al.*, 2014).

This study evaluated the efficiency of systematic roguing of diseased plants, through weekly inspections, as an efficient and long-lasting means of managing the disease in the field. In addition, the latent period (time from inoculation to when the host becomes a source of inoculum) and incubation period (time from inoculation to onset of symptoms) of CABMV in passion flower were determined, to optimize the efficiency of the roguing.

Materials and methods

Greenhouse experiments

Plant material and virus isolates

Healthy passion fruit plants of variety FB-200 were obtained from Flora Brasil® seeds sown in 1.5 L pots containing a mixture of autoclaved soil, sand and manure. At the 1–2-true-leaf stage, the seedlings were transplanted to 1.5 L pots containing the same substrate, with two seedlings per pot. The seedlings were watered daily and a fertilizer formulation of NPK (10:10:10) was applied at periodic intervals.

Three CABMV isolates collected from field-infected passion flowers representing the states of São Paulo (SP), Rio de Janeiro (RJ) and Bahia (BA), Brazil, were used in the experiments. These isolates were named CABMV-SP, CABMV-RJ and CABMV-BA, respectively. The isolates were maintained in passion fruit plants and periodically renewed through mechanical inoculation to new plants. The identity of the isolates was confirmed by reverse transcription (RT)-PCR, using CABMV-specific primers that amplify part of the HC-Pro gene (Correa *et al.*, 2015).

Mechanical inoculation

Leaves from passion fruit plants infected with each CABMV isolate were macerated separately in 0.02 M potassium phosphate buffer, pH 7, plus 0.02 M sodium sulphite, at a ratio of 1:20 (w:v). The mechanical inoculation was performed on the second true leaf of the test plants, which were previously dusted with carborundum abrasive. The inoculum was applied with a porcelain pestle moistened with plant extract and rubbed four times on each leaf. The leaves were then washed with tap water to remove excess inoculum and abrasive.

PTA-ELISA

CABMV in the leaf tissues was detected by plate-trapped antigen (PTA)-ELISA (Mowat & Dawson, 1987). Leaf tissue was diluted 1:20 in extraction buffer (carbonate, pH 9.6). Polyclonal antiserum against CABMV, produced in the Laboratory of Plant Virology, ESALQ/USP, was used. The antiserum was diluted 1:1000, and alkaline phosphatase-conjugated immunoglobulin (Sigma A-8025) was diluted 1:32 000 in phosphate-buffered saline, pH 7.4. Leaves of healthy and CABMV-infected plants were used as negative and positive controls, respectively. Samples were tested in duplicate. Absorbance values (405 nm) were measured in a Metertech Σ960 ELISA reader, 60 min after addition of diluted *p*-nitrophenyl phosphate (0.5 g mL^{-1}) in diethanol-amine buffer. Samples were considered positive when the mean absorbance value was at least three times higher than the mean absorbance value of the negative control.

Evaluation of latent and incubation periods of CABMV in passion flower

Passion fruit plants at the 3–4-true-leaf stage were mechanically inoculated with isolates CABMV-SP, CABMV-BA or CABMV-RJ, separately. These plants were subsequently used as sources of inoculum in transmission assays performed at different time intervals: 3, 4, 5 and 6 days after inoculation (DAI). Four plants inoculated with the respective CABMV isolates were used as sources of inoculum at each time interval. Leaf extracts from each plant were mechanically and separately inoculated on healthy test plants at the 3–4-true-leaf stage. All the plants used in the experiment were maintained in a greenhouse under ambient photoperiod and room temperature. The latent period of CABMV in passion flower was determined by confirming virus transmission to 32 test plants of each isolate, by observing the symptoms and detecting the virus by PTA-ELISA. The experiment was repeated twice.

To assess the incubation period of CABMV, 20 plants at the 3–4-true-leaf stage were mechanically inoculated with isolates CABMV-SP, CABMV-BA or CABMV-RJ, separately. The incubation period was determined by daily monitoring of CABMV-induced symptoms in the inoculated plants, up to 30 DAI. At the end of the evaluations, all the inoculated plants were analysed for the presence of CABMV by PTA-ELISA. The experiment was repeated twice.

Field experiments

Location and description of experiments

Experiments were carried out from 2013 to 2018 in the states of São Paulo and Bahia, Brazil. In São Paulo, the experiments were conducted in three areas located at the 'Luiz de Queiroz' School of Agriculture, in the municipality of Piracicaba. These areas were denoted SP-X (22°41′30.11″S, 47°38′40.45″W), SP-Y (22°41′37.01″S, 47°38′28.76″W) and SP-Z (22°42′27.57″S, 47°37′52.29″W). The areas SP-X and SP-Y were 400 m apart, and approximately 2.5 km from area SP-Z. In Bahia, the experiments were conducted in the municipality of Vitória da Conquista, in two areas located on Bela Vista farm and in another area belonging to the State University of Southwestern Bahia. These areas were denominated BA-X (14°54′56.5″S, 40°49′21.3″W), BA-Y (14°54′56.5″S, 40°49′21.8″W) and BA-Z (14°53′20″S, 40°48′03″W). Areas BA-X and BA-Y were 3 m apart, and approximately 3.8 km from area BA-Z.

Transplanting and maintenance of plants in the field

Passion flower seedlings of variety FB-200 were grown in 2.6 L plastic bags containing a mixture of autoclaved soil, manure and sand. The seedlings were maintained in a greenhouse until they reached 80–120 cm height and were transplanted in the field in pits previously prepared and filled with 200 g plain superphosphate, 100 g calcium and 1 L earthworm humus. Fertilizers were applied based on recommendations for raising the crop.

Two support systems were used for the vines: trellises (Fig. 1a) and individual arbours (Fig. 1b). The trellises consisted of a row of posts 2 m tall and 5 m apart, supporting a smooth metal wire extended between the end posts. The vines were trained along the wire. The distance between trellises was 4 m in São Paulo and 3 m in Bahia. The spacing between seedlings along the trellis rows was 5 m in São Paulo and 2 m in Bahia. On the trellises, the plants were trained in two ways: (i) with individual plants allowed to interlace with neighbouring plants, as in commercial plantations; and (ii) with the plants separated by training the ends of the vines back along the wire, or by pruning the vines to prevent interweaving between neighbouring plants. The individual arbours were composed of two T-shaped wooden structures, 1.5 m apart. From the centre and each side of the T-cross pieces, three straight metal wires were extended to train the plants. In the experiments carried out in São Paulo, the spacing between arbours was 3 m between rows and 1.5 m within each row, and in Bahia the spacing was 3 × 1 m.

Roguing of diseased plants

Experiments in São Paulo

In São Paulo the experiments were conducted with treatments named A, B and C (Table 1). The first experiment compared treatments A and C. Treatment A consisted of 100 healthy passion flowers transplanted in area SP-X on 26 September 2013. The plants were set out and trained separately on arbours. After transplantation, the plants were inspected weekly and those with symptoms of CABMV infection were immediately eradicated. Treatment C (control) consisted of 56 passion flowers transplanted in area SP-Z on 10 January 2014. The plants were set out and trained on a strand of wire, with no separation of plants. They were inspected periodically, but CABMV-infected plants were not removed.

The second experiment compared treatments A, B and C (Table 1). The plants in these treatments were transplanted in areas SP-X, SP-Y and SP-Z, respectively, on 16 and 18 November 2016. Treatments A and B used 97 passion fruit plants each. The plants in these treatments, which were trained separately on arbours and wire trellises, respectively, were inspected weekly and those with symptoms of CABMV infection were removed. Treatment C (control) consisted of 54 plants cultivated in area SP-Z, on a wire trellis with no separation of plants. In this area the infected plants were identified and confirmed periodically, but not eradicated.

In both experiments, all the plants infected with CABMV in treatment C (control) and those that were removed in treatments A and B had the presence of the virus confirmed by PTA-ELISA.

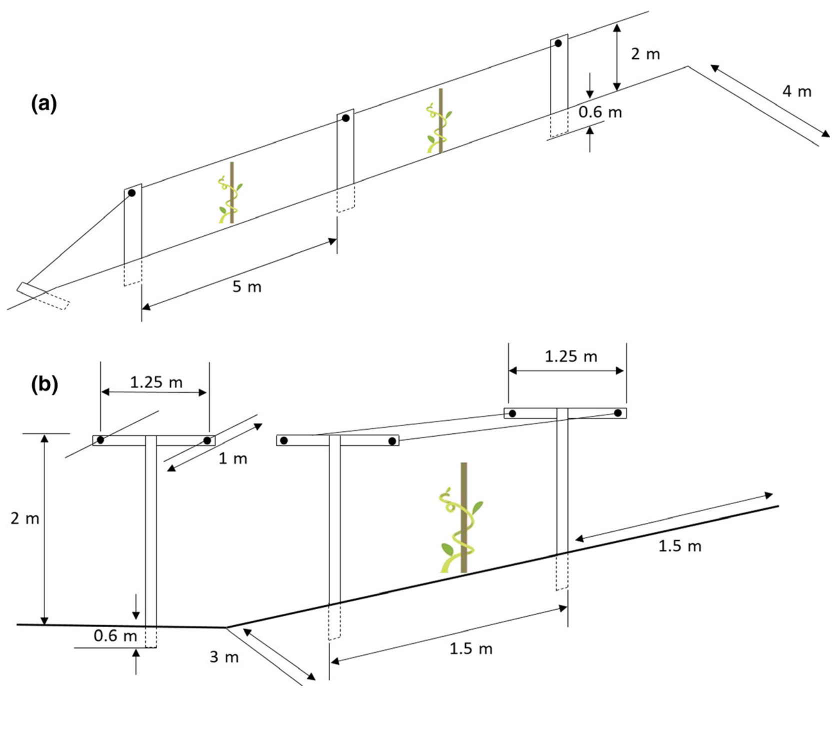

Figure 1 Illustration of trellis with wire (a) and individual arbour (b).

Table 1 Planting areas and respective treatments.

Treatment	Area (m^2)	Plant support system	Diseased plants removed
A	SP-X (900)	Arbour, plants separated	Yes
B	SP-Y (1600)	Trellis, plants separated	Yes
C	SP-Z (450)	Trellis, plants not separated	No (control)
D	BA-X (750)	Arbour, plants separated	Yes
E	BA-Y (480)	Trellis, plants separated	Yes
F	BA-Z (480)	Trellis, plants not separated	No (control)

Identification of diseased plants was based on visual inspection of early symptoms, around the arbours and on both sides of the trellises.

Experiments in Bahia

In Bahia the experiments were performed with treatments named D, E and F (Table 1). The first experiment was carried out in areas BA-X and BA-Z, comparing treatments D and F, respectively. In area BA-X, 100 plants were transplanted on 11 November 2013. The vines were trained individually on arbours, inspected weekly, and those with symptoms of CABMV-infection were immediately removed. In the control area BA-Z, where no systematic removal of diseased plants was carried out, 102 plants were transplanted on traditional wire trellises on 28 September 2013, with no separation of plants.

The second experiment was carried out in the same areas BA-X and BA-Z. The seedlings were transplanted to the field on 20 September 2014. The number of plants and training methods were the same as in the first experiment.

The third experiment was conducted in areas BA-X, BA-Y and BA-Z, comparing treatments D, E and F, respectively. One hundred plants were transplanted in area BA-X and 107 plants in area BA-Y, on 11 March 2017. In area BA-X the vines were trained on individual arbours and in BA-Y on wire trellises, with the vines kept separate by pruning back the branches of neighbouring vines on the same wire. These areas were inspected weekly and plants with symptoms of CABMV-infection were eradicated. As a control, in area BA-Z, 130 plants were transplanted on 20 December 2016 and trained on traditional wire trellises, without separation of plants. These were inspected periodically but infected plants were not eradicated.

In all three experiments, for all vines infected with CABMV in treatment F (control) and those that were removed in treatments D and E, the presence of the virus was confirmed by PTA-ELISA. The plants were inspected visually as described before.

Statistical analysis

Latent and incubation periods

For the purposes of this analysis, the state of a passion fruit plant acting as a source of inoculum or not consisted of a binary response: Y = 1 or 0, respectively. Logistic regression analysis was performed using generalized linear models with a binomial distribution family for modelling the 0/1 response against days after CABMV inoculation, and CABMV isolates interaction. Experiment replicate was included as blocking factor (not interacting with the other two predictors). The fitted curves were compared for each CABMV isolate and the latent period was estimated as the day at which 50% of passion fruit plants acted as sources of inoculum.

Survival analysis was used for a suitable statistical technique for modelling time-to-event variables, as the period from a pathogen inoculation to presence of disease symptoms (defined as incubation period). The advantage of this analysis technique is that it can accommodate cases in which the event has not occurred by the end of the study (30 days in the present study). Cox proportional hazards regression models were fitted to the time (in days) from the inoculation until the appearance of CABMV typical symptoms, and Kaplan–Meier plots were done for each CABMV isolate.

Efficacy of roguing for managing CABMV in passion flower

Generalized linear model was used to assess the efficacy of roguing of CABMV-infected plants in passion flower experimental fields. Binary logistic function was fitted to model the relationship between plant status (0 = noninfected, 1 = infected) against days post-transplant, including disease roguing treatment as covariate, obtaining the logit(p), where p is the probability of a plant being infected by CABMV. The fitted models for each individual experiment (combination of location × year) estimated a linear predictor via a specific link function (logit, in this study) including the effect of days from transplanting, the disease roguing treatment and their interaction. A significant interaction indicates that the rate of disease progress differs between treatments, which is a question of special interest in this analysis. By back-transforming the fitted values to the proportion scale, the predicted incidences were calculated as a function of the days and type of CABMV roguing, and plotted for a better understanding of the results.

The statistical analyses of data and model plotting results were conducted using R (R Core Team, 2019). The GLM function from the STATS package was used to fit the binary data and predict function for back-transforming the estimated logit(p) to probability scale. The significance of the predictor variables (days, treatment, and their interaction) were tested in the deviance analysis by the Wald statistic z-value using the ANOVA function in the CAR package (Fox & Weisberg, 2011). GLM diagnostics were inspected with DHARMA package (Hartig, 2019) and linear predictor comparisons were performed with the estimable function from GMODEL package (Warnes *et al.*, 2019). LOGISTIC DISPLAY function from EPIDISPLAY package (Chongsuvivatwong, 2018) was used to estimate the odds ratio results from logistic models.

The COXPH and SURVFIT functions from SURVIVAL package (Therneau, 2015) were used to fit the survival curves and Cox proportional hazards regression models, respectively. For a better comprehension of results, the survival curves were transformed to their cumulative event plots by $f(y) = 1-y$. The GGSURVPLOT function from the SURVMINER package was used to plot the survival curves (Kassambara & Kosinski, 2019).

Results

CABMV latent and incubation periods in passion flower

The shortest latent period of isolates CABMV-SP and CABMV-BA in the passion fruit plants was 3 DAI, and 4 DAI for isolate CABMV-RJ (Table S1). Complementary studies carried out with these isolates indicated that prior to 3 DAI, inoculated plants did not serve as a source of viral inoculum in attempts to mechanically transmit CABMV to other healthy plants (data not shown). For all viral isolates, the number of plants used as sources of inoculum and which allowed transmission of CABMV to other plants increased with the time after inoculation.

During the evaluation period for isolate CABMV-SP, a maximum of 44% of plants acted as source of inoculum (Fig. 2a). For isolates CABMV-BA and CABMV-RJ, the time when 50% of plants acted as sources of inoculum was 4.5 and 4.6 DAI, respectively. Both isolates reached a maximum of 93% of plants acting as sources of inoculum at 6 DAI (Fig. 2a).

The incubation periods of the CABMV isolates in the 120 plants evaluated ranged from 4 to 15 DAI (Table S2). The symptom onset in 50% of the infected plants was not different among isolates ($P = 0.4$) and was estimated at 7 DAI for CABMV-SP and CABMV-BA, and 8 DAI for CABMV-RJ (Fig. 2b).

Roguing of diseased plants

In the first experiment in São Paulo state, for treatment SP-X, only 16 CABMV-infected plants were removed during 2 years of training the vines separately on the arbours. For the control treatment (SP-Z), where no roguing was performed, the first infected plant was identified 102 days after transplanting (DAT). Eighty-nine days later all plants in treatment SP-Z were infected with the virus (Fig. 3a).

In the second experiment, for treatments SP-X and SP-Y, the first infected plants were identified at 192 and 203 DAT, respectively (Fig. 3b). Thereafter, diseased plants were eradicated weekly. At approximately 240 DAT, new infected plants were observed and eradicated in these treatments. Subsequently, infections in plants of both treatments recurred weekly, despite the continuous roguing of diseased plants. Due to the high number of infected plants in these treatments, roguing was suspended at 281 DAT, and 90 days later all plants were infected (Fig. 3b). All plants in treatment SP-Z (control), where diseased plants were not removed, were infected with CABMV at 129 DAT (Fig. 3b).

In the first experiment in the state of Bahia, for area BA-X, 54 plants were eradicated within 120 days, whereas for area BA-Z (control), 100% of the plants were infected during this period (Fig. 3c). As the number of eradicated plants was high, the experiment was interrupted. In the second experiment, within 160 days all plants in area BA-Z, where roguing was not carried out, were infected. In the same period, only nine plants were removed in area BA-X (Fig. 3d).

In the third experiment, the first infections in areas BA-X and BA-Y occurred at 28 and 59 DAT, respectively. By 240 DAT, approximately 50% of the plants had been eradicated (Fig. 3e), which led to the suspension of roguing. In less than 2 months after roguing was halted, all passion fruit plants were infected with CABMV. For area BA-Z, where roguing was not performed, at 125 DAT all plants were infected (Fig. 3e).

The interaction between days after transplanting and roguing of diseased plants was significant for modelling CABMV incidence in all individual experiments, therefore the CABMV incidence varied with the treatment. Consistently, roguing practice, either on an arbour or

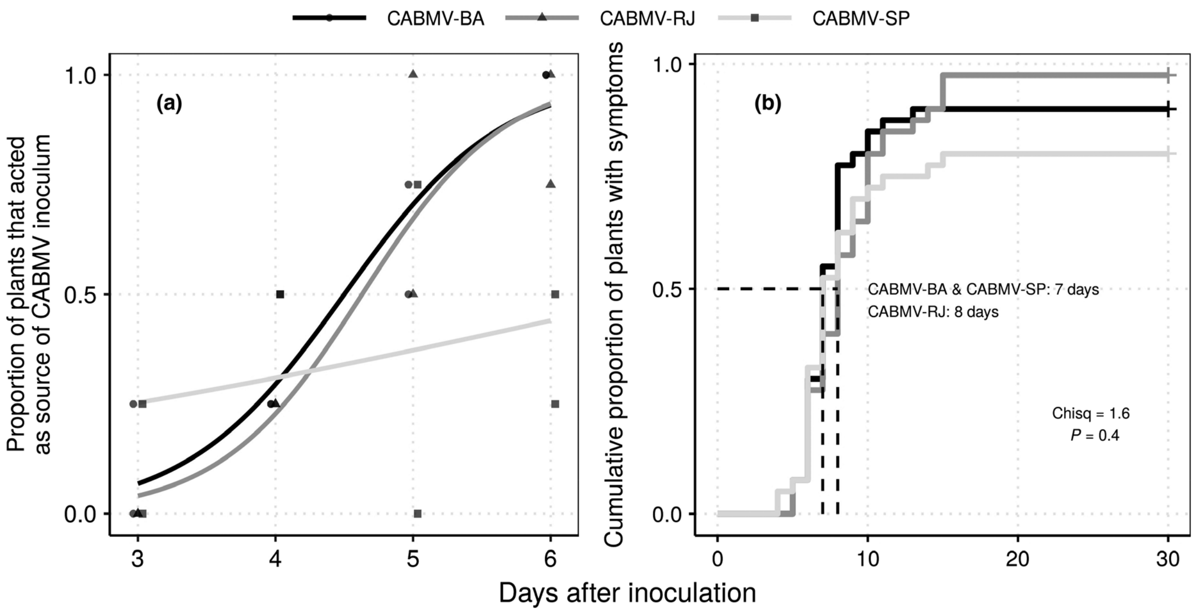

Figure 2 Latent (a) and incubation period (b) for CABMV isolates from Bahia (BA), Rio de Janeiro (RJ) and São Paulo (SP), Brazil, inoculated on passion fruit plants. (a) Estimated probability (obtained by logistic regression analysis) of passion flowers acting as source of inoculum as a function of days after CABMV inoculation (fitted curves) and experimental observations. (b) Cumulative hazard curves (obtained by inverse transformation of the Kaplan–Meier estimate) for the time at which an inoculated passion fruit plant showed typical symptoms.

trellis system, significantly reduced the CABMV incidence progress relative to the control plots in which the infected plants were not removed (Table 2). No differences were observed in the CABMV incidence between roguing diseased passion fruit plants on an arbour or trellis training system (Table 2).

Discussion

The latent and incubation periods are epidemiological parameters that must be taken into account in the management of plant diseases (Rimbaud *et al.*, 2015). Incubation periods that are shorter than latent periods indicate that only hosts with symptoms may act as sources of inoculum, whereas incubation periods that are longer than latent periods indicate that symptomless hosts may already act as sources of inoculum and contribute to the spread of the pathogen in the field (Chan & Jeger, 1994). The former situation is more common for fungal pathogens, while the latter occurs more frequently for bacteria and viruses (Vanderplank, 1975). Therefore, management of plant disease by roguing will be more effective when there is a synchronism between the latent and incubation periods.

The results of the present study showed that, in general, the passion fruit plants inoculated with CABMV started to act as sources of inoculum from 3 DAI, and showed symptoms, on average, at 8 DAI. The percentage of virus transmission from the plants used as sources of inoculum to the test plants was highest (75%) at 6 DAI. The incubation periods of the CABMV

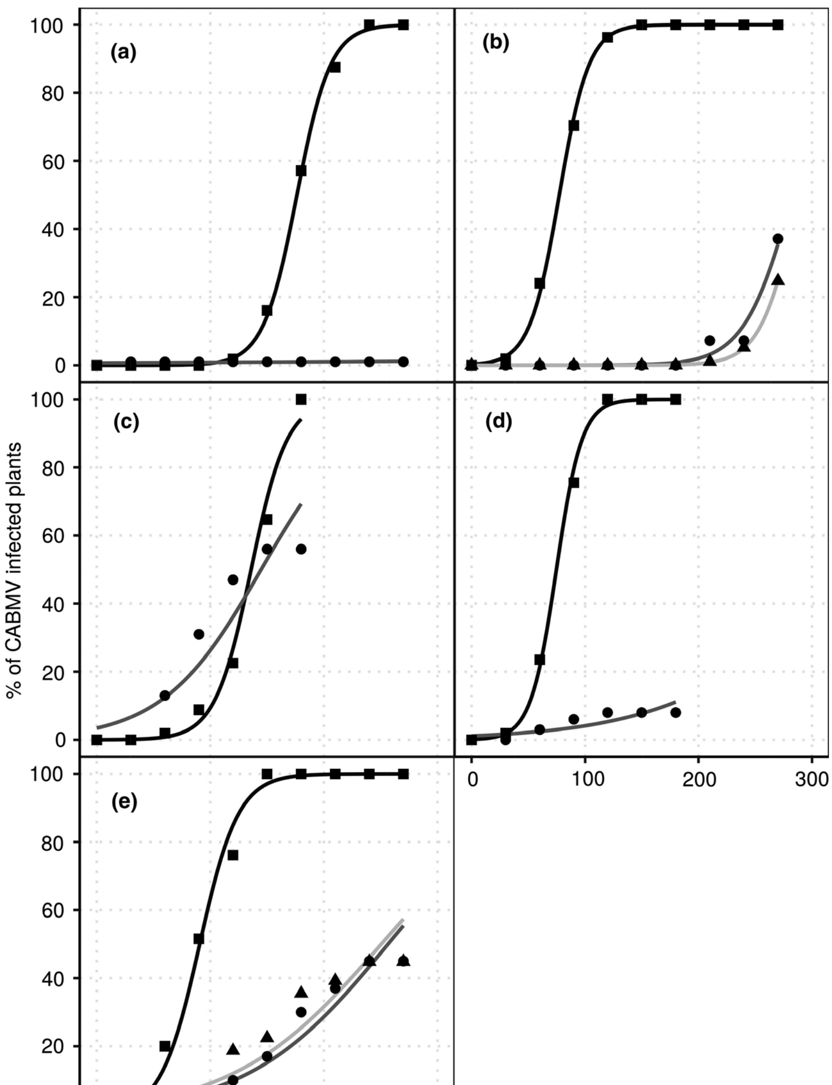

Figure 3 Cumulative percentage of passion fruit plants infected with CABMV (individual observations and fitted curves) in the control areas (squares and black line) and diseased rogued plants in the arbours (circles and grey lines) and trellises with separated plants (triangles and light grey lines). (a, b) São Paulo 2013 and 2016, respectively; (c, d, e) Bahia 2013, 2014 and 2016, respectively.

isolates ranged from 4 to 15 DAI, with a mean of 8 DAI. For all three virus isolates combined, 77% (80/104) of the inoculated plants expressed symptoms within an interval of 4–8 DAI. The lag time between the incubation and latent periods of CABMV in passion flower is approximately 2 days. Results of one assay (data not show), performed with CABMV-SP transmitted by the aphid *M. persicae*, were similar to that obtained by mechanical transmission. The average of latent and incubation periods were 8 and 9 days, respectively. Rimbaud *et al.* (2015) also reported a short time interval between the incubation and latent periods of plum pox virus (PPV) in peaches (*Prunus persica*). The symptoms induced by this potyvirus appeared between 6 and 13 DAI and the plant became infectious 1 day after the symptoms appeared. Considering that the mean incubation period of CABMV in passion fruit plants was 8 days, it is recommended that orchards be inspected at least once a week, so that diseased plants can be identified and eradicated to effectively reduce the sources of inoculum and consequently the secondary spread of the pathogen in orchards.

Infected passion fruit plants were identified in the field based on expression of the initial disease symptoms, characterized by vein clearing and mild leaf mottling. Confirmation of the infection by PTA-ELISA in 100% of the eradicated plants showed that the procedure adopted was efficient. This same criterion proved to be efficient in the application of annual roguing to manage grapevine leafroll-associated virus 3 in plantings of red-berried grapevine cultivars in New Zealand and South Africa (Bell *et al.*, 2017). The authors pointed out that of the 114 782 vines evaluated visually for symptoms and indexed by ELISA, the two methods agreed for 114 701 (99.9%) of the plants.

Roguing is a cultural practice that has been used successfully for more than 30 years to control papaya ringspot caused by the potyvirus papaya ringspot virus-type P (PRSV-P) in the Brazilian states of Bahia and Espírito Santo (Rezende & Kitajima, 2018). Management of citrus huanglongbing, caused by the bacterium '*Candidatus* Liberibacter asiaticus', through the removal of infected trees, has also proved to be efficient in controlling the disease in citrus orchards, when carried out regionally and combined with the control of the vector (Belasque *et al.*, 2010; Bassanezi *et al.*, 2013). Other diseases in which roguing has been adopted as a control strategy include those caused by citrus tristeza virus (CTV), banana bunchy top virus (BBTV) and cocoa swollen shoot virus (CSSV) (Allen, 1978; Fishman *et al.*, 1983; Thresh & Owusu, 1986).

Passion fruit woodiness disease has epidemiological characteristics similar to that of papaya ringspot disease. The potyviruses causing both diseases are transmitted nonpersistently by several species of aphids, which do not colonize plants of these two species. Both potyviruses also have a limited host range. In the case of papaya ringspot, only papaya is the principal source of PRSV-P inoculum. Although this potyvirus can infect some species of Cucurbitaceae, they do not appear to be important in the epidemiology of papaya ringspot (Mansilla *et al.*, 2013; Spadotti *et al.*, 2013). In the case of passion flower, there is evidence that the sources of inoculum appear to be mainly the two cultivated species (*P. edulis* and *P. alata*) and some wild *Passiflora* species, reported as susceptible to CABMV (Maciel *et al.*, 2009).

The main difference between the papaya and passion fruit plants, which affects the application of roguing of diseased plants, is their architecture. The papaya is an erect, unbranched tree, while the passion flower is a woody vine, which attaches to its support by means of tendrils and intertwines with neighbouring vines. Therefore, after the plants interweave, even though the symptoms may be apparent, it is laborious to determine which is the infected plant to be rogued. To overcome this difficulty, two methods of separating the vines were applied to facilitate recognition of infected plants and permit their immediate eradication. Both these separation procedures allowed the diseased plants to be easily identified and removed. The strategies to separate plants trained on a wire can be easily adopted, because trellises are the conventional system used by growers in commercial passion flower orchards in Brazil. In addition, trellises are more economical than installing arbours.

Table 2 Comparison of CABMV incidence progress curves in passion flower fitted by logistic regression of five experiments conducted in São Paulo (2013 and 2016) and Bahia (2013, 2014 and 2016), Brazil.

			Contrasts of fitted curves (*P*-value)[d]		
Experiment[a]	McFadden's pseudo-R^{2}[a]	DAT × CABMV-RT (*P*-value)[c]	Control vs Arbour	Control vs Trellis	Arbour vs Trellis
SP 2013	0.99	<0.001	1.7×10^{-6}	—	—
SP 2016	0.90	0.025	9.2×10^{-5}	3.1×10^{-4}	0.235
BA 2013	0.92	<0.001	3.7×10^{-12}	—	—
BA 2014	0.98	<0.001	0.004	—	—
BA 2016	0.96	<0.001	0.011	0.001	0.389

[a]Experiment location and year.

[b]McFadden's pseudo-R^2: the closer to 1, the better the covariates can predict the outcome.

[c]Significant ($P < 0.05$) DAT (days after transplanting) × CABMV-RT (roguing treatment) interaction effect means that at least one curve is different.

[d]Significant ($P < 0.05$) contrasts of linear predictor (both estimated coefficients: intercept and slope) mean that curves of two compared CABMV roguing treatments are different.

Although arbours greatly facilitate the separation of plants and consequently the application of roguing, they are more expensive than trellises.

The efficiency of roguing in the management of passion fruit woodiness disease is also directly associated with the presence of external sources of inoculum close to the planting areas. These sources are plants in old and abandoned orchards, small farms and backyards (Fischer & Rezende, 2008), and some CABMV-susceptible wild species of *Passiflora* (Maciel *et al.*, 2009) in small patches of woods near the plantations. This was established in the first experiment conducted in Bahia, where the large number of eradicated plants (56% in 4 months) was attributed to the presence of wild passion flower in small forests close to the experimental field, which probably functioned as constant primary sources of the pathogen. After most of these infected wild passion fruit plants were removed prior to the implementation of the second experiment, the number of infected and rogued plants in the same area were reduced to only 8% over a period of 180 days. The higher number of rogued plants in the second experiment in São Paulo can also be attributed to the presence of possible external sources of inoculum. Despite an intensive search, these sources of inoculum were not identified in the vicinity of the experimental area. Notably, in the first experiment in this area in the state of São Paulo, only 16% of the plants were rogued, and the plants remained viable for 25 months. In all five control plantings where roguing was not performed, after the first infected plant was noticed, 100% of the plants were infected within approximately 120 days.

Management of diseases by roguing can have two benefits. The first is the increased useful life of healthy plants, by reducing secondary spread of the pathogen in the planting through eradicating local sources of inoculum. The second is that dead or less-productive infected plants can be replaced with healthy ones, when appropriate, which can compensate for the loss of yield (Sisterson & Stenger, 2013). In the passion flower orchards, replacing eradicated plants may be feasible provided that some healthy seedlings are permanently maintained in nurseries.

Taken together, the results indicate that systematic roguing of diseased plants can be recommended for managing passion fruit woodiness, provided it is applied on a regional scale by all passion fruit growers. In order to facilitate the identification and subsequent removal of infected plants, it is necessary to grow the vines separately on wire trellises or on individual arbours. It is necessary that the technicians responsible for identifying and eradicating infected plants be properly trained to promptly recognize the initial symptoms of the disease. The reduction of external sources of inoculum is also an indispensable measure that must be adopted by the growers, so that management of passion fruit woodiness through roguing of diseased plants attains the desired efficiency. The development of some pilot plantings for the application of roguing in a passion flower-producing region is recommended to validate the use of this technique for managing passion fruit woodiness disease.

Acknowledgements

This study was supported by Fundação de Amparo à Pesquisa no Estado de São Paulo (FAPESP), project nos. 2009/18612-7 and 2016/08573-8, and PNPD/CAPES 02427/09-5. This article does not contain any studies with human participants or animal performed by any of the authors. The authors thank Felipe Franco de Oliveira and Luis Fernando Sanglade Marchiori for technical help.

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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. Latent periods of CABMV isolates evaluated by mechanical transmission to passion fruit plants with sources of inoculum at different intervals (days) post-inoculation.

Table S2. Incubation periods (days) of isolates CABMV-SP, CABMV-RJ and CABMV-BA inoculated mechanically into 20 passion fruit plants and evaluated for 30 days.