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Selection of pathometric variables to assess resistance and infectivity in the passion fruit woodiness pathosystem

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Abstract The objective of this work was to compare the efficiency of four pathometric variables, i.e., grading scale, infection intensity index, global leaf disease index, and global incidence and severity index, in evaluations of the passion fruit/*Cowpea aphid-borne mosaic virus* (CABMV) pathosystem. In 'yellow' passion fruit plants, mechanically inoculated with CABMV isolates, the degree of association between leaf symptoms and the total fruit yield was determined, as well as the relative infectivity between viral isolates. Only infection intensity index and the global incidence and severity index presented significant regressions with yield (p=0.007 and 0.006, respectively). These two pathometric variables could also distinguish groups of viral isolates inducing different severity levels (0.001 and 0.002, respectively). The results indicate that infection intensity index and global incidence and severity index are equally efficient in early identification of passion fruit populations with a higher resistance/susceptibility level to CABMV, and can be helpful in identifying more severe CABMV isolates, both useful aids in breeding programs.

Keywords Disease index · Grading scale · *Passiflora edulis* · Tolerance

Abbreviations

CABMV	*Cowpea aphid-borne mosaic virus*
GISI	Global incidence and severity index
GLDI	Global leaf disease index
GS	Grading scale
III	index of infection intensity
PV	Pathometric variable

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Introduction

The yellow passion fruit plant (*Passiflora edulis* Sims) is the most cultivated *Passiflora* species in the world, and it predominates in the Brazilian market (Alexandre et al. 2004; Bellon et al. 2007). Brazil stands out among the world's producers of passion fruit, producing 429,253 t in an area of 37,310 ha (Brasil, 2005). However, Brazil's average production of 10.43 t/ha (Brasil 2005) is considered low when compared to the potential productivity of the crop, which can reach 40–50 t/ha under optimal cultivation conditions (Ruggiero 1987; Meletti et al. 2005).

The occurrence of pests and pathogens causes yield and quality damage and consequently production losses (Meletti et al. 2005). Among Passion fruit, the passion fruit woodiness disease (Kitajima et al. 1986), which in Brazil is caused by the *Cowpea aphid-borne mosaic virus* (CABMV) (Nascimento et al. 2004; Moreira 2008), is one of the most important, and has been reported in several other countries (McKern et al. 1993; Sithole-Niang et al. 1996; Iwai et al. 2006). In Brazil, it was first recorded in the 1970s in the State of Bahia (Yamashiro and Chagas 1979).

Research on the reaction of passion fruit to CABMV is limited in native species of *Passiflora* (Maciel et al., 2009) and in 'yellow' passion fruit (Leão et al. 2006), although there are many records regarding its occurrence and general control recommendations (Kitajima et al. 1986; Chagas et al. 1992). Damage and loss evaluation is scarce (Gioria et al. 2000; Junqueira et al. 2003; Cerqueira-Silva et al. 2008). In order to reduce losses, identification of resistant cultivars is critical for passion fruit breeding (Meletti et al. 2005). However, this research field has not been adequately explored for Brazilian cropping systems (Meletti et al. 2000; Nascimento et al. 2003; Farias et al. 2005a, b).

It is common to use descriptive keys to quantify symptoms and to employ a disease index in evaluations of disease severity (Laranjeira 2005; Moraes 2007). The uses of grade scales and disease indices (Novaes and Rezende 1999; Junqueira et al. 2003; Nascimento et al. 2006; Cerqueira-Silva et al. 2008) are examples of this application for the *Passiflora*/CABMV pathosystem as well as for other pathosystems (McKinney 1923; Czermainski 1999; Santos et al. 2009). The characterization of passion fruit species reactions through a grading scale has been carried out in association with serological evaluations of virus genotypes (Novaes and Rezende 1999). Also, the determination of percentage of leaf area affected (Leão et al. 2006), or other pathometric variables (PV) has been used (Gioria et al. 2000).

Considering the importance of pathometry in the evaluation of the plants' reaction to pathogens (Czermainski 1999; Laranjeira 2005), this work aimed to compare the efficiency of PVs in the passion fruit/CABMV pathosystem and to identify: (*i*) passion fruit genotypes differing in terms of resistance/susceptibility to CABMV, and (*ii*) CABMV isolates with different degrees of infectivity.

Materials and methods

Biological material

The experiments in this study used adult plants and seedlings. Adult 'yellow' passion fruit plants were obtained from the germination of seeds from open pollinated fruits. 'Yellow' passion fruit seedlings were obtained from the germination of seeds from the production region of Livramento de Nossa Senhora, Bahia State, Brazil.

Virus isolates utilized in this study (i.e., UESB-01 to UESB-11), were from plants with typical woodiness symptoms collected in production region of Livramento de Nossa Senhora, Bahia. All isolates exhibited a high percentage of identity (> 93 %) with the coat protein amino acid sequences of CABMV strains available in GenBank (Moreira 2008; Cerqueira-Silva et al. 2008). However it is not possible to rule out the possibility of multiple virus infections, since the virus isolates utilized were collected from plants of passion fruit localized in a production region.

All 'yellow' passion fruit (plants and seedlings) were mechanically inoculated with infective sap. This procedure was carried out three times, at 60, 75 and 90 days after germination, using extracts prepared in 5 % (w/v) potassium phosphate buffer (0.02 M), pH 7.0, from leaf samples of woodiness symptomatic plants.

Selection of variables to assess resistance—Assay A

Assay A compared pathometric variables (PVs) for the identification of the degree of resistance/susceptibility between passion fruit plants to CABMV. It comprised 87 treatments (one treatment=one 'yellow' passion fruit genotype) and 87 experimental units (one replication by treatment; one replication=one adult ‘yellow’ passion fruit plant) and was set up in the *Passiflora* research field, Vitória da Conquista, Bahia State, of the Universidade Estadual do Sudoeste da Bahia (UESB), adopting the growing conditions of Bruckner and Picanço (2001).

Plant reactions to inoculation with CABMV were measured at 150 days after the last inoculation time, using four PVs: (*i*) a grading scale (GS) with four levels, where 0 = no symptoms, 1 = mild mosaic without leaf distortion, 2 = severe mosaic without leaf distortion, and 3 = severe mosaic, blisters and leaf distortion, from the descriptive key of Novaes and Rezende (2003); (*ii*) an index of infection intensity (III), varying from 0 to 100, as described by Silva (1969), and generalized for discrete or quantitative ordinal scales by Czermainski (1999); (*iii*) a global leaf disease index (GLDI), varying from 0 to 1, as described by Cerqueira-Silva et al. (2008); and (*iv*) a global incidence and severity index (GISI), varying from 0 to 3, and calculated to two decimal places by the following formula:

$$GISI = \frac{\sum GS \times I}{NDL} - \left(1 - \frac{NDL}{TNL}\right)$$

where SI = incidence sum multiplied by the GS, NDL = number of diseased leaves, and TNL = total number of leaves. In the calculations the pathometric variables III, GISI and GLDI used the symptomatologic classification based on the GS. Variable GISI is described for the first time in the present study. Except for variable GS, all the leaves of 'yellow' passion fruit plants were evaluated. The total weight of the fruits (TWF) was also determined.

Determination of relative severity at CABMV isolates—Assay B

Assay B compared PVs for identification of degree of infectivity among CABMV isolates, using ‘yellow’ passion fruit seedlings from the production region of Livramento de Nossa Senhora, Bahia. It comprised 11 treatments [one treatment = one CABMV isolate, the last treatment being the control (water)] and 110 experimental units (10 replications by treatment, one replication = one ‘yellow’ passion fruit seedling) and was conducted at a UESB greenhouse, Vitória da Conquista, Bahia State.

The yellow passion fruit seedling reactions to inoculation with the CABMV were measured at 60 days after the last inoculation time, using the same PV described for Assay A.

Statistic analyses

The average values of the PVs measured, for assays A and B, were used to estimate the standard error, coefficient of variation, normality (Lilliefors test) and kurtosis. Specifically for assay B, analysis of variance and tests of comparison of the means (Student-Newman-Keuls ‘SNK’) were employed to identify the infectivity degree of CABMV isolates. Analysis was performed using BioEstat 4.0 software (Ayres et al. 2005), with α=0.05.

For assay A, the observed values of the PVs had their significance of association with fruit yield evaluated through linear correlation tests, curve fitting for determination of best fit regression, bilateral Student’s *t*-test for comparison of means, and comparison of coefficients of variation tests. For the regression tests performed, the PV was considered the predictor variable and fruit yield the dependent variable. Dispersion analysis for the production of fruit in grams and the different PVs was performed to determine the inherent variability of productivity and reaction to CABMV among the 87 individuals.

Based on the high or low severity and fruit yield among the 87 plants, two contrasting samples of the

Table 1 Statistical and descriptive parameters of pathometric variables measured in a population of 87 ‘yellow’ passion fruit plants, mechanically inoculated with CABMV isolate UESB-01 and evaluated under field conditions

Variable	Range	Average	Kurtosis	Standard error	Coefficient of variation
GS	0–3	2.74	−0.162	0.05	16 %
III	0–100	51.40	−0.051	0.05	10 %
GLDI	0–1	0.61	−0.020	0.01	16 %
GISI	0–3	2.73	−0.824	0.60	16 %

GS = grading scale; III = index of infection intensity; GISI = global index of incidence and severity; GLDI = global leaf disease index

Table 2 Magnitude and significance of *p*-values of the normality/Lilliefors and linear correlation tests between pathometric variables and productivity, measured in a population of 87 'yellow' passion fruit plants, mechanically inoculated with CABMV isolate UESB-01 and evaluated under field conditions

Variable	*p*-value	
	Normality[a]	TWF
GS	Abnormal**	0.13
III	Normal[ns]	0.008
GLDI	Normal[ns]	0.008
GISI	Abnormal**	0.40

[a] Values of the Lilliefors normality test

TWF total weight of the fruits, *ns* not significant

** Significant at the 1 % level of probability. See Table 1 for abbreviations

populations, one sample of nine passion fruit genotypes with high resistance and one sample of nine passion fruit genotypes with high susceptibility to CABMV, adopting a 10 % selection intensity (i.e., percentage of the number of individuals in relation to all individuals under study). The efficiency in the selection of these contrasted samples was validated by means of Student's *t*-test and bootstrapping with 10,000 re-samplings.

Results and discussion

Selection of variables to assess resistance—Assay A

For assay A, the average results of GS and GISI (~2.7) were near to the maximum value (3) of their respective scales (Table 1). On the other hand, the estimated values of III and GLDI (51.4 and 0.61, respectively) were close to the mid-values of their respective scales. GS and GISI tended to classify the individuals as highly susceptible which was confirmed by the kurtosis values obtained (−0.162 and −0.824, for GS and GISI, respectively), as opposed to those estimated for III and GLDI (−0.051 and −0.020, respectively). These results may indicate that GS and GISI overemphasized the high degree of severity. Considering that viral particles of *Potyviridae* are not uniformly distributed in the plant tissues and organs (Novaes and Rezende, 2003), it can be inferred that GS and GISI fail to detect degrees of resistance/susceptibility among individuals of a passion fruit plant population. The variables GS and GISI rate the plants according to the most prominent leaf symptom detected, not taking into consideration how the passion fruit as a whole responds to the multiplication and movement of CABMV in its organs. Therefore, due to the fact that

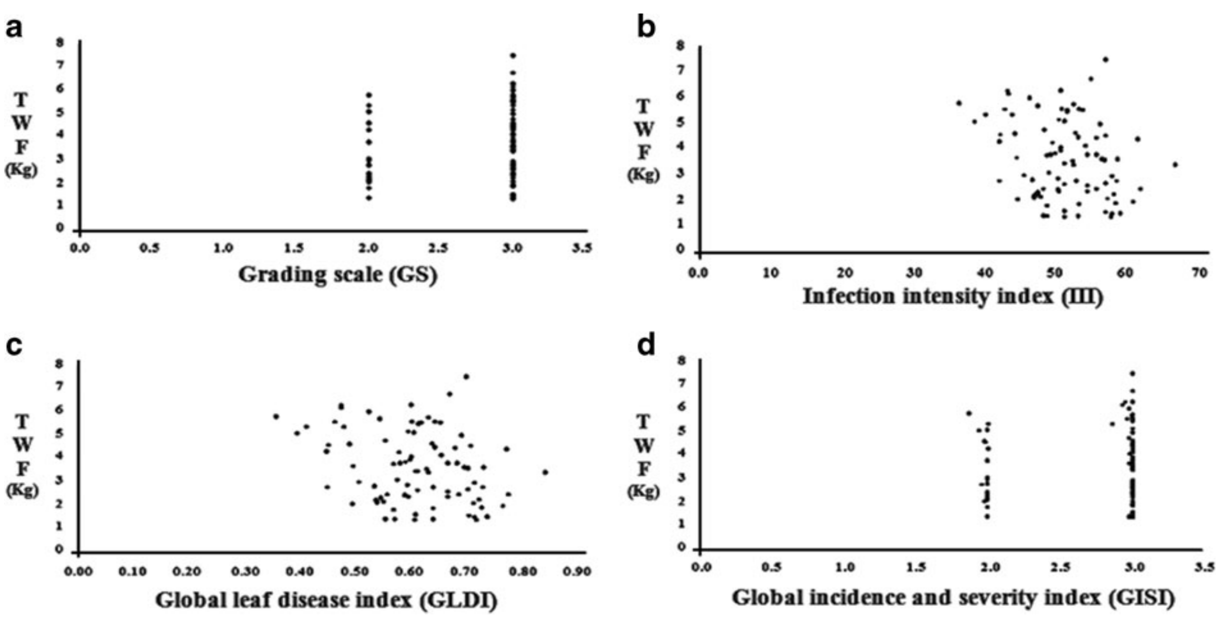

Fig. 1 Dispersion graphics of 87 'yellow' passion fruit plants, mechanically inoculated with CABMV isolate UESB-01 and maintained under field conditions. Total weight of the fruits and severity of the fruit woodiness disease were evaluated through: 'GS' varying from 0 to 3 (**a**); 'III' varying from 0 to 100 (**b**); 'GLDI' varying from 0 to 1 (**c**); and 'GISI' varying from 0 to 3 (**d**)

they are highlighted extremes, the variables GS and GISI fail to detect "mild relationships" between variation in the foliar symptoms and the weight of fruit.

The magnitude of PV coefficientd of variation was similar (10–16 %) but variable III differed from the other PVs ($p<0.001$; comparison of coefficients of variation test). This result corroborates the proposition of Czermainski (1999) that III reduces the variability of symptomatologic evaluations.

Inverse and significant correlations ($p=0.008$; validated by bootstrapping) were detected between III and GLDI versus TWF ($r=-0.278$ and -0.282, respectively; Table 2). The fact that GS and GISI estimates (Fig. 1) had very low variability resulted in non-significant correlations between these PVs and TWF [$p=0.13$ (GS) and 0.40 (GISI)]. Therefore, these variables are not efficient for distinguishing the degree of resistance/susceptibility that may exist in a given population of passion fruit plants.

Leão et al. (2006) reported that GS evaluation, associated with the percentage of symptomatic leaf area, allowed the statistical detection of individuals susceptible and resistant to CABMV. However, they did not evaluate or link fruit yield to GS (Leão et al. 2006).

The dependency of TWF regarding to III and GLDI was evaluated through a fitted curve test (Table 3). These two PVs were selected, because they presented a significant inverse correlation with TWF (Table 2). It was determined that a logarithmic regression explains the relationship between III or GLDI and TWF ($p=0.007$ and $R^2=8.15$ % for III versus TWF; $p=0.006$ and $R^2=8.51$ % for GLDI versus TWF) (Table 3) variables. These results corroborate those obtained though the correlation test, suggesting that symptom intensity in leaves correlates with yield losses. The

Table 3 Magnitude of R2 values obtained from regressions determined through curve fitting test, among the pathometric (predictive) and productivity (dependent) variables, measured in a population of 87 'yellow' passion fruit plants, mechanically inoculated with CABMV isolate UESB-01 and evaluated under field conditions

Regressions evaluated	Predictive and dependent variables	
	III x TWF	GLDI x TWF
Linear	7.77 % **	7.78 % **
Exponential	7.12 % *	7.31 % *
Logarithm	8.15 % **	8.51 % **
Geometric	7.47 % *	7.79 % **

*Values significant at 5 % level of probability

**Values significant at 1 % level of probability

See Tables 1 and 2 for abbreviations

Table 4 Magnitude of p-values between pathometric and productivity variables of two contrasting samples of populations of 'yellow' passion fruit plants on the reaction to CABMV, determined via bilateral Student's *t*-test and bootstrap test

Variable	Contrasted samples of population			
	Higher[a]	Lower[b]	*t*-test[c]	Bootstrap[d]
III	41.1	60.3	0.0001*	0.0001
GLDI	0.43	0.75	0.0001	0.0001
TWF	5.1	2.7	0.0001	0.0001

[a] Sample of nine genotypes with low severity of symptoms

[b] Sample of nine genotypes with higth severity of symptoms

[c] *p*-values obtained in the bilateral *t*-test

[d] *p*-values obtained through bootstrapping with 10,000 replications

*Considered significant at the 1 % level of probability

See Tables 1 and 2 for abbreviations

Table 5 Pathometric variables averages measured in a sample of 10 yellow passion fruit plants, mechanically inoculated with CABMV isolates UESB-02 to UESB-11 and evaluated in the greenhouse

Viral isolate	Pathometric variable	
	III[(1)]	GLDI[(1)]
UESB 02	54.1 a	0.51 ab
UESB 03	37.3 ab	0.46 ab
UESB 04	30.2 ab	0.37 ab
UESB 05	23.2 b	0.34 ab
UESB 06	31.3 ab	0.40 ab
UESB 07	32.0 ab	0.39 ab
UESB 08	38.0 ab	0.41 ab
UESB 09	47.4 a	0.50 a
UESB 10	39.8 a	0.50 a
UESB 11	26.2 b	0.27 b
Control (distilled water)	1.7 c	0.03 c
Average	35.95	0.41
Standard error	2.9	0.02
Coefficient of variation	26.3 %	18.8 %

[(1)] Values in the same column featuring different letters are statistically different ($p<0.05$; SNK test comparison of the means). See Table 1 for abbreviations damage can be explained, in part, by the destruction of chloroplasts in the symptomatic regions, acting negatively on the photosynthetic potential of the plant (Chaves 2002; Santos et al. 2004).

Dispersion analysis shows that III and GLDI reveal a high genetic variability regarding reaction to CABMV and fruit yield among the 87 genotypes (Fig. 1). The low dispersion of GS and GISI related to TWF (Fig. 1a and d) is due to the fact that these two PVs poorly distinguish the reaction between passion fruits plants. By contrast the higher dispersion of III and GLDI estimates (Fig. 1b and c) shows that these variables are better able to distinguish the reaction between passion fruits plants in relation to the leaf symptoms of CABMV.

Variables III and GLDI presented statistically different mean results (*t*-test, $p<0.0001$; bootstrap, $0.0001<p<0.007$) between the two contrasting samples of 'yellow' passion fruit plants to CABMV infection (Table 4). These results support the hypothesis that III and GLDI can be used to differentiate statistically different populations (taken to be putative 'resistant' and 'highly susceptible') concerning their fruit yield. The sample of 'resistant' genotypes presented an increase in TWF of 89 % compared to the one of 'highly susceptible' genotypes (Table 4).

Determination of relative severity at CABMV isolates —Assay B

For assay B, comparing PVs in the determination of the relative severity of CABMV isolates, mean results of the leaf disease symptoms enabled the identification of isolates with different levels of severity (Table 5). Both variables III and GLDI identified two statistically different groups ($p=0.001$ and 0.002, respectively; SNK test comparison of the means). These groups were formed by isolates of greater severity (UESB 02, 09 and 10) and lesser severity (UESB 05 and 11) for III, and isolates of greater severity (UESB 09 and 10) and lesser severity (UESB 05) for GLDI.

The isolates characterized in this study with respect to the relative severity are a new resource that might be useful for further research which aims to characterize wild germplasm by using less severe isolates (such as the UESB 05), or to assess the level of resistance of cultivated varieties by using more severe isolates (09 UESB and 10). Considering the genetic variability of CABMV and PWV isolates worldwide, the variables III and GLDI provide evidence of an effective contribution to a possible standardization of methods in the characterization and selection of viral isolates.

Overall, our data for assays A and B indicate that III and GLDI are good tools to characterize and select initial populations for passion fruit breeding programs targeting resistance to woodiness disease. Also, they can differentiate CABMV isolate infectivity. Hence, they are helpful to select both mild and severe isolates for using in breeding programs.

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