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ARTICLE

Yellow passion fruit reaction to *Xanthomonas axonopodis* pv. *passiflorae* and to *Cowpea aphid-borne mosaic virus*

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Abstract: *Yellow passion fruit (Passiflora edulis Sims) yield and longevity have been drastically reduced by bacterial spot (Xanthomonas axonopodis pv. passiflorae - Xap) and passion fruit woodiness disease (PWD) (Cowpea aphid-borne mosaic virus - CABMV). This study was aimed at evaluating the reaction of 11 genotypes of yellow passion fruit, based on the reaction of their progenies, to both mechanically inoculated Xap and CABMV, under greenhouse conditions. There was a progressive increase in bacterial spot and PWD severity with time. BRS Gigante Amarelo, MAR20#12, and MAR20#34 were selected as the progenies with lowest bacterial spot severity and disease progress over time. MAR20#2005, EC-L-7, UnB2015-1, and EC-3-0 presented the lowest PWD severity and disease progress over time. These progenies, along with individual plants from other progenies in which disease severity was significantly low until the last evaluation, will be cloned and tested again for Xap and CABMV, including other isolates.*

Key words: *Bacterial spot disease, passion fruit woodiness disease, genetic breeding.*

INTRODUCTION

Brazil is the largest passion fruit producer in the world. *Passiflora edulis* Sims (yellow passion fruit) stands out as the predominant species, corresponding to 95% of Brazil´s production (Costa et al. 2008), whereas BRS Gigante Amarelo (BRS GA1) is one of the major cultivars planted due to its high fruit and pulp yields, good fruit quality, and disease resistance (Meletti 2011). The reported passion fruit annual production is 703,489 ton within a harvested area of 49,889 ha (IBGE 2016). Nevertheless, based on the productive potential of the species, especially under experimental conditions, Brazilian passion fruit yield (14,101 kg ha^{-1}) is still considered low (Freitas et al. 2011).

In Brazil, passion fruit is infected by many plant pathogens, which can compromise fruit quality, reduce yield, and shorten the crop's productive cycle. Among them, bacterial spot disease, caused by *Xanthomonas axonopodis* pv. *passiflorae* (Xap), and passion fruit woodiness disease (PWD), caused by *Cowpea aphid-borne mosaic virus* (CABMV), are of great importance to Brazilian passion fruit production (Carvalho et al. 2015).

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The lack of high yield and pathogen resistant genotypes is a limiting factor to fruit quality and fruit yield increase. Moreover, high disease susceptibility showed by yellow passion fruit has already decreased crop production and planted area in important producing states in Brazil (Faleiro and Junqueira 2016). For this reason, there is an increased effort, through breeding programs, in order to obtain genotypes that show, both, superior agronomic characteristics and resistance to the main diseases (Faleiro and Junqueira 2016, Silva et al. 2017). Resistant genotypes could reduce yield losses caused by plant pathogens. Therefore, classification of genotype reaction to infection by pathogens based on descriptive keys and disease ratings are of great importance in breeding programs (Cerqueira-Silva et al. 2014). Disease quantification can be estimated mainly by disease incidence and severity with the aid of descriptive keys or diagrammatic scales (Silva and Michereff 2016).

Reliable evaluations require a large number of plants per trial, which makes greenhouse screening a valuable tool as part of a breeding program (Kososki et al. 2008, Maciel et al. 2009). However, no data regarding multiple disease resistance in this species have been reported to date. The objective of this study was to evaluate 11 genotypes of yellow passion fruit, based on the reaction of their progenies, to both Xap and CABMV, under greenhouse conditions.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse complex (14 – 30 ºC; 61-82% ARH) at University of Brasília's (UnB) Experiment Station (lat 16º S, long 48º W, alt 1010 m asl), located in Brasilia, DF, Brazil. The genotypes evaluated were obtained from research studies developed by Embrapa (Empresa Brasileira de Pesquisa Agropecuária – Brazilian Agricultural Research Corporation) and UnB, which used yield, fruit quality, and disease resistance as selection criteria (J. R. Peixoto, pers. comm.). Genotypes MAR20#12, MAR20#24, MAR20#34, MAR20#49, and MAR20#2005 were originated from mass selection from nine superior genotypes: Maguary Mesa 1, Maguary Mesa 2, Havaiano, MSC (Marília Seleção Cerrado), Seleção DF, EC-2-0, F1 (Marília x Roxo Australiano), F1 (Roxo Fiji x Marília), and RC1 [F1 (Marília x Roxo Australiano) x Marília (recurrent parent)]. BRS GA1 is a comercial hybrid cultivar and UnB2015-1 was originated from mass selection of 32 genotypes at UnB's Água Limpa Farm (FAL). MSCA is derived from cultivar Marília Seleção Cerrado whereas EC-3-0 is a hybrid (RC1) obtained from controlled pollination between Marília x Roxo Australiano cultivars backcrossed with Marília (F1 x Marília). EC-L-7 is derived from cultivar Marília and Yellow Master FB200 is a commercial cultivar.

Seeds were sown in November 2013, in polystyrene trays (120 mL cell^{-1}) filled with Vivatto Slim Plus® (Technes Agrícola Ltda) artificial substrate. Seedlings were transplanted in July 2014 into 2 L plastic bags filled with soil. They were daily watered (400 mL) and fertilized every two weeks with urea (0.1 g plant^{-1} at each fertilization event), until experiment initiation. The fertilizer was dissolved in water prior to application directly to the substrate. No pest control was performed during the trial.

The experiment consisted of inoculating passion fruit plants with Xap on 30 January 2015, followed by inoculation with CABMV on 15 May 2015, on the same plants. Bacteria inoculation was performed using the Xap strain registered as UnB-1393, which was obtained from the plant pathology laboratory at UnB. The UnB-1393 strain was multiplied using 523 culture media (Kado and Heskett 1970) at 28–30 ºC, for 72 h (Franco and Takatsu 2004). The bacterial suspension concentration (~1 x 10^6 CFU mL^{-1}) was adjusted on a spectrophotometer to an optical density of 0.145 at 550 nm wavelength, previously determined by the calibration curve. For inoculation, four needles were simultaneously immersed in the bacterial suspension and then used to pierce the adaxial leaf surface of three leaves per plant (adapted from Viana et al. 2014a). After inoculation, plants were kept in a humid chamber for 72 h. Disease incidence was calculated as the percentage of plants infected and disease severity was calculated as the percentage of total leaf area with necrotic lesions. Disease incidence and severity were recorded at a 7-day interval after disease symptoms first appeared. The first evaluation was performed 12 days after inoculation. For disease severity assessment, a 0 to 5 scale (adapted from Viana et al. 2014a) was used, as follows: 0 – no symptoms; 1 – 1 to 10% of total leaf area with necrotic lesion; 2 – 11 to 25% of total leaf area with necrotic lesion; 3 – 26 to 50% of total leaf area with necrotic lesion; 4 – more than 50% of total leaf area with necrotic lesion; and 5 – leaf drop. Based on the average of the severity scores (AS) obtained from this scale, plants were classified as: Resistant (R), $0 \leq AS < 1$; Moderately Resistant (MR), $1 \leq AS < 2$; Moderately Susceptible (MS), $2 \leq AS < 3$; Susceptible (S), $3 \leq AS < 4$; and Highly Susceptible (HS), $AS \geq 4$ (Viana et al. 2014a). At the end of the bacterial spot disease assessments, plants were pruned and they were fertilized every two weeks until CABMV inoculation.

The CABMV isolate was collected from yellow passion fruit plants in UnB's Água Limpa Farm (FAL). Three young leaves per plant were inoculated with the CABMV isolate, by gently rubbing the adaxial foliar surface with a vegetable extract obtained from the maceration of leaves of yellow passion fruit showing severe symptoms of CABMV infection, including mosaic, leaf deformations and blisters. The leaf macerate was diluted 1:2 (weight:volume) with a buffer solution (0.1 M potassium phosphate + 0.1 M sodium sulphite), pH 7.0, with addition of a few grams of silica (Celite® 503; Sigma-Aldrich Co.) as described by Viana et al. (2014b). Plants were washed 10 min after inoculation in order to avoid leaf burn due to silica abrasiveness. Disease incidence (% of plants infected) and disease severity (leaf symptoms) were recorded at 7-day intervals after disease symptoms first appeared. The first of five evaluations was performed 21 days after inoculation. For disease severity assessment, a 1 to 4 scale (Viana et al. 2014b) was used, as follows: 1 – no symptoms; 2 – mild mosaic and no leaf deformation; 3 – mild mosaic, blisters and leaf deformation; 4 – severe mosaic, blisters and leaf deformation. The score per plant was defined based on the most severe symptoms observed in the leaves. Based on the AS obtained from this scale, plants were classified as: Resistant, $1 \leq AS \leq 1.5$; Moderately Susceptible, $1.5 < AS \leq 2.5$; Susceptible, $2.5 < AS \leq 3.5$; Highly Susceptible, $3.5 < AS \leq 4$ (adapted from Viana et al. 2014b).

The experiment consisted of a randomized block design (RBD) with subdivided parcels comprised of 11 treatments (genotypes), four repetitions, six replications per genotype, and five evaluations. Analysis of variance was performed to evaluate possible interactions between progeny and evaluation date. Regression analysis was performed to evaluate linear and quadratic responses of progenies to evaluation dates. The means were grouped by the Scott-Knott´s test ($P \leq 0.05$). Disease severity and incidence heritability, genetic and environmental coefficient of variation ratio (GCV/ECV), and phenotypic correlations between disease severity and incidence were calculated. Correlation intensity was classified according to the magnitude of the values, as suggested by Carvalho et al. (2004): $r = 0$ (null); $0 < |r| \leq 0.30$ (weak); $0.30 < |r| \leq 0.60$ (medium); $0.60 < |r| \leq 0.90$ (strong); $0.90 < |r| \leq 1$ (very strong); and $|r| = 1$ (perfect). The area under the disease progress curve (AUDPC) was calculated using AS score data collected in the five evaluation dates, according to Campbell and Madden (1990). AUDPC was used as an attempt to differentiate progenies regarding their resistance to bacterial spot and to woodiness virus diseases. The means were grouped by the Scott-Knott´s test ($P \leq 0.05$). All analyses were performed using Genes software (Cruz 2013).

RESULTS AND DISCUSSION

Bacterial spot severity assessments identified an interaction between progenies and evaluation dates ($P \leq 0.05$). Progenies differed for mean disease severity although no differences between progenies were found within evaluations one, four, and five (Table 1). These evaluations corresponded to the very initial and to the maximum disease development stages, respectively, and therefore no difference was expected to be found. Disease severity assessments demonstrated that BRS GA1 was the progeny with the lowest score (3.47) whereas EC-L-7 showed the highest disease severity (3.90).

Table 1. Bacterial spot disease severity and resistance degree in yellow passion fruit (*Passiflora edulis* Sims) mechanically inoculated with *Xanthomonas axonopodis* pv. *passiflorae*

Progeny	Evaluations					Severity (AS)	Resistance Degree
	1	2	3	4	5		
MAR20#12	1.05 aA	3.54 bB	4.09 aC	4.66 aD	4.92 aD	3.65 b	S
MAR20#24	1.03 aA	3.68 bB	4.46 bC	4.85 aD	5.00 aD	3.80 c	S
MAR20#34	1.08 aA	3.09 aB	4.22 aC	4.92 aD	4.96 aD	3.65 b	S
MAR20#49	1.22 aA	3.65 bB	4.25 aC	4.72 aD	4.93 aD	3.76 c	S
MAR20#2005	1.13 aA	3.36 aB	4.33 aC	4.80 aD	5.00 aD	3.72 c	S
BRS Gigante Amarelo	0.93 aA	2.85 aB	3.99 aC	4.72 aD	4.86 aD	3.47 a	S
UnB2015-1	1.30 aA	3.76 bB	4.42 bC	4.81 aD	4.93 aD	3.84 c	S
MSCA	0.95 aA	3.75 bB	4.59 bC	4.92 aC	5.00 aC	3.84 c	S
EC-3-0	1.17 aA	3.59 bB	4.30 aC	4.81 aD	5.00 aD	3.77 c	S
EC-L-7	1.20 aA	4.08 bB	4.64 bC	4.74 aC	4.85 aC	3.90 c	S
Yellow Master FB200	1.04 aA	3.95 bB	4.49 bC	4.73 aC	4.86 aC	3.81 c	S

AS = Average of severity scores of 5 evaluations. Mean grouping in columns by Scott-Knott´s test. Different lowercase letters within columns and uppercase letters within rows indicate significant differences ($P \leq 0.05$).

According to the mean number obtained from the grading scale, all progenies were classified as susceptible (Table 1). After five bacterial spot disease severity assessments, none of the progenies had a single resistant plant. Studies have pointed towards a difficulty in distinguishing genotypes for disease resistance. Meanwhile, it is important to recognize that in breeding programs with focus on disease resistance, any minimal difference between and within progenies or genotypes is useful in providing information for resistance selection (Kudo et al. 2012, Fuhrmann et al. 2014).

Additionally, there were differences among evaluations ($P \leq 0.05$), revealing a progressive increase in bacterial spot severity with time. Assessments one to four differed for disease severity except for progenies EC-L-7, Yellow Master FB200, and MSCA, which showed no difference between assessments three and four (Table 1). These progenies proved to be more susceptible, reaching high severity scores early during evaluation three. At the fifth evaluation, disease severity had already achieved its maximum and did not differ from the fourth evaluation for any progeny. No differences were found among progenies and among evaluation dates for bacterial spot incidence ($P > 0.05$).

Bacterial spot severity and incidence increase with time were estimated by quadratic regression (Figure 1). It was observed that maximum bacterial spot severity was achieved before evaluations had finished for all progenies studied. Maximum bacterial spot severity was observed on progenies Yellow Master FB200 and EC-L-7, 21 days after the first evaluation, and on progenies MSCA, MAR20#24 and UnB2015-1, 22 days after the first evaluation. Progenies MAR20#34 and BRS GA1 showed maximum disease severity 25 and 26 days after first evaluation, respectively.

Differences among progenies and among evaluation dates were found for PWD severity ($P \leq 0.05$). MAR20#2005 stood out as the progeny with the lowest PWD severity (1.75) whereas BRS GA1 (2.08) presented the highest score for disease severity estimates. It was observed an increase in PWD severity with time, revealing differences among all five severity assessments and among the first four incidence assessments. Based on the mean number obtained from the grading scale, progenies were classified as moderately susceptible (Table 2). However, after five evaluations, resistant plants could be observed in all progenies. EC-L-7 (41%) and MAR20#12 (33%) were the progenies with the greatest numbers of resistant plants at the end of the study, contrasting with BRS GA1 (11%) and MAR20#24 (9%) (Table 2).

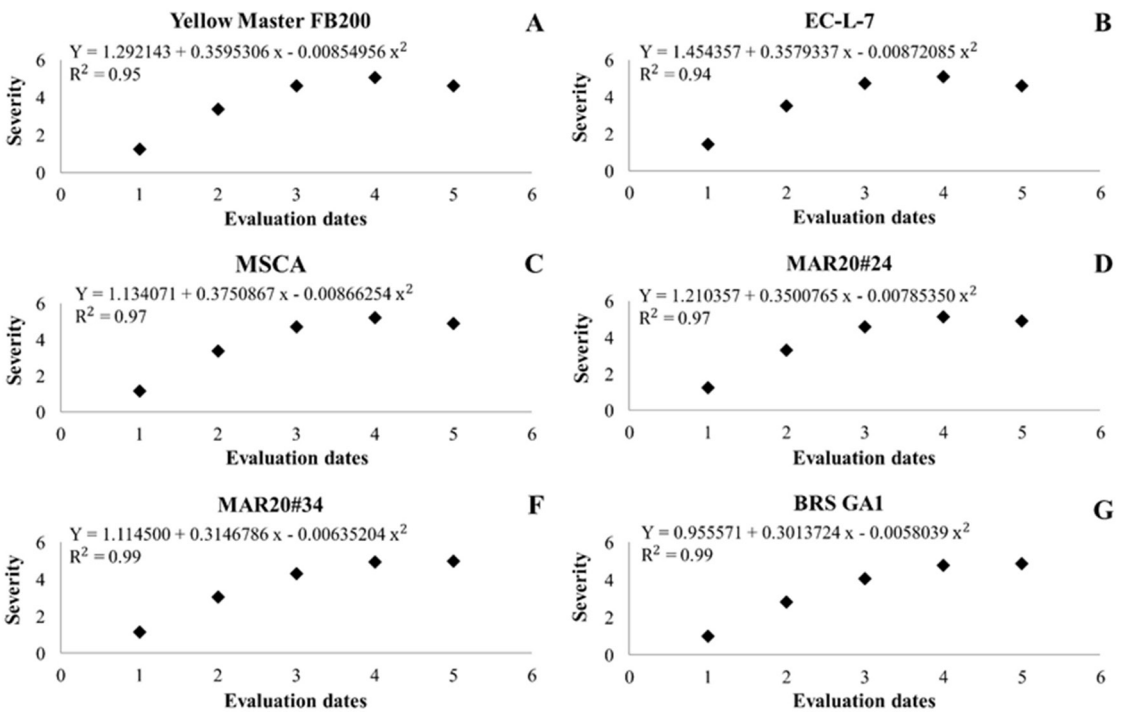

Figure 1. Bacterial spot disease severity increase over time in yellow passion fruit (*Passiflora edulis* Sims) mechanically inoculated with *Xanthomonas axonopodis* pv. *passiflorae*.

Table 2. Passion fruit woodiness virus disease (PWD) severity, resistance degree, and percentage of resistant plants in yellow passion fruit (*Passiflora edulis* Sims) mechanically inoculated with *Cowpea aphid-borne mosaic virus* (CABMV)

Progeny	Evaluations					Severity (AS)	Resistance Degree	%R
	1	2	3	4	5			
MAR20#12	1.60 aA	1.74 aA	2.14 aB	2.37 aB	2.47 aB	2.07 b	MS	33
MAR20#24	1.28 aA	1.59 aA	2.41 aB	2.46 aB	2.59 aB	2.06 b	MS	9
MAR20#34	1.34 aA	1.73 aA	2.10 aB	2.36 aB	2.41 aB	1.99 b	MS	10
MAR20#49	1.36 aA	1.60 aA	2.19 aB	2.34 aB	2.42 aB	1.98 b	MS	19
MAR20#2005	1.19 aA	1.44 aA	1.88 aB	1.94 aB	2.31 aB	1.75 a	MS	25
BRS Gigante Amarelo	1.27 aA	1.77 aB	2.27 aC	2.46 aC	2.62 aC	2.08 b	MS	11
UnB2015-1	1.39 aA	1.48 aA	1.91 aB	2.19 aB	2.28 aB	1.85 a	MS	29
MSCA	1.38 aA	1.60 aA	2.31 aB	2.44 aB	2.44 aB	2.03 b	MS	18
EC-3-0	1.11 aA	1.60 aB	2.08 aC	2.25 aC	2.37 aC	1.88 a	MS	28
EC-L-7	1.37 aA	1.55 aA	1.85 aB	2.02 aB	2.23 aB	1.81 a	MS	41
Yellow Master FB200	1.29 aA	1.85 aB	2.23 aC	2.42 aC	2.50 aC	2.07 b	MS	26

AS = Average of severity scores of 5 evaluations. %R = Percentage of resistant plants at the end of the study, 49 days after inoculation. Mean grouping in columns by Scott-Knott´s test. Different lowercase letters within columns and uppercase letters within rows indicate significant differences ($P \leq 0.05$)

According to Junqueira et al. (2003), disease resistance variability in yellow passion fruit is low, but it may occur within plants of the same cultivar (Fuhrmann et al. 2014).

PWD severity and incidence increase with time were estimated by linear and quadratic regression, respectively. Our results indicate that the disease severity had not achieved its maximum at the end of the evaluations (Figure 2A), emphasizing the need of a greater number of evaluations over time. Maximum PWD incidence was observed 27 days after first disease assessment and before evaluations had finished (Figure 2B), similarly to data previously reported by Viana et al. (2014b).

In contrast to the results here described for the bacterial spot disease, Viana et al. (2014a) reported that genotype MSCA was resistant to the bacterial spot disease whereas MAR20#12, Yellow Master FB200, EC-L-7, and EC-3-0 were moderately resistant. Differences were detected among genotypes regarding disease incidence. Furthermore, Pinto et al. (2008) indicated that genotypes MAR20#12, MAR 20#24, and MAR20#34 were resistant to PWD, differing from the results reported in this study (Table 2).

Divergences between our results and those reported in the literature were expected due to the lack of systematization shown by the different studies. For example, Viana et al. (2014a) evaluated the response of 18 genotypes (12 plants per genotype) to the UnB-767 Xap strain, during four evaluation dates. The 6-month-old plants were inoculated in the abaxial leaf surface using a 10^8 UFC mL^{-1} bacterial suspension. After inoculation, plants were kept in a humid chamber for 24 hours. In another study, Pinto et al. (2008) evaluated the response of 62 genotypes (4 plants per genotype) to CABMV during only two evaluation dates. The 4-month-old plants were inoculated with an isolate collected in Araguari, MG. The first evaluation was performed 30 days after inoculation and the second evaluation was performed 15 days after first disease assessment. Fertilization was performed immediately after the first disease evaluation. Therefore,

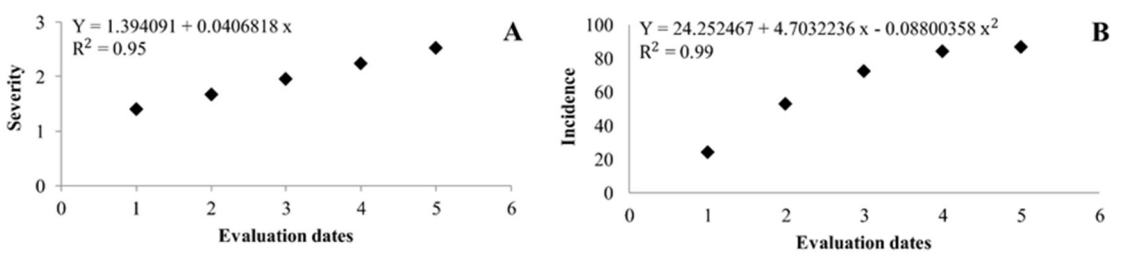

Figure 2. Passion fruit woodiness disease (PWD) severity (A) and incidence (B) increase over time in yellow passion fruit (*Passiflora edulis* Sims) mechanically inoculated with *Cowpea aphid-borne mosaic virus* (CABMV).

the differences observed could be explained by the distinct inoculation methods and inoculum concentrations used; plant growth conditions (i. e., space available for root growth); plant age at inoculation; plant nutritional status; climate conditions during the period studied; number of plants studied; and number of evaluations performed. Disease progress can be influenced by several factors, such as plant resistance, pathogen aggressiveness, and climate conditions. Hence, evaluations must be carried out over a certain period of time, which varies according to the disease being evaluated, in order to obtain reliable data on the disease development (Kososki et al. 2008, Viana et al. 2014a). A lower number of evaluations could lead to a misleading classification of genotypes into a specific resistance category.

The susceptibility of progenies to Xap and to CABMV observed in our study could also be due to plant variability and/or to the isolates' aggressiveness. Differences in diseased leaf area demonstrated genetic variation and segregation, allowing for the identification of resistance genes to the bacterial spot disease in yellow passion fruit (Lopes et al. 2006). Considerable genetic variability in the resistance of yellow passion fruit to CABMV has also been described by Oliveira et al. (2013) and Cerqueira-Silva et al. (2015). Moreover, analysis of genetic diversity among Xap and CABMV isolates reported existence of variability for aggressiveness within and among Xap populations (Nakatani et al. 2009), as well as genetic variability among CABMV isolates (Melo et al. 2015). This information supports the possibility of existing pathogen-host interactions, which could lead to different responses from genotypes to pathogen isolates (Lopes et al. 2006, Fuhrmann et al. 2014). Therefore, it is essential to consider plant and pathogen genetic variability as well as pathogen aggressiveness when establishing strategies for a breeding program.

AUDPC is a useful measurement of disease intensity over time. It entails repeated disease assessments and allows for characterization of plant-pathogen-environment interactions (Simko and Piepho 2012). In this study, AUDPC was calculated for disease severity. However, no differences were found among progenies for bacterial spot and PWD ($P > 0.05$).

Medium heritability values were observed for bacterial spot severity (38%) and AUDPC (47%). GCV/ECV was only 0.39 and 0.47 for disease severity and AUDPC, respectively. These data suggest that there is low genetic variability within genotypes and/or environmental conditions were not favourable for selection, once genetic variance was lower than environmental variance. As disease progress can be influenced by the environment, it is possible that the adoption of traditional breeding methods could result in low efficiency on improving passion fruit resistance to bacterial spot. Thus, breeding methods based on family performance would be more appropriate than those based on individual plants performance (Alves et al. 2006).

One of the main goals of a breeding program focusing on disease resistance is to obtain resistant genotypes to multiple diseases. Naturally occurring multiple resistance to various pathogens has been reported for a few crops, such as bean (Melo et al. 2008), maize (Wisser et al. 2011), and potato (Neder et al. 2010). Nonetheless, no multiple pathogen reaction data is available for yellow passion fruit to date. Our results not only differentiated progeny response to bacterial spot disease and to PWD, but also indicated correlations between the diseases tested. The trait bacterial spot incidence was highly and negatively correlated to PWD incidence (-0.79; $P \leq 0.01$) and severity (-0.73; $P \leq 0.05$). Passion fruit woodiness disease incidence was positively correlated to PWD severity at magnitude of 0.76 ($P \leq 0.05$). There was a contrasting response from progenies regarding bacterial spot disease and PWD. In general, progenies with greater resistance to bacterial spot showed greater susceptibility to PWD. Further studies with multiple pathogen inoculation are necessary in order to better understand plants response to different diseases, especially to PWD, which lacks conclusive results.

Our findings are in accordance with previous studies performed with yellow passion fruit in Brazil. It has been observed that BRS GA1 and other genotypes may present low degrees of severity for several diseases, such as anthracnosis and bacterial spot, but they still present susceptibility to PWD (Oliveira et al. 2013). Our data strengthens the importance of continuous PWD studies in order to allow for meaningful decision-making and for efficient use of vector and disease control methods.

As a result of this study, all genotypes were considered susceptible to bacterial spot, but BRS GA1, MAR20#12, and MAR20#34 were selected as the progenies with the lowest disease ratings (Table 1). All materials were also considered moderately susceptible to PWD, but MAR20#2005, EC-L-7, UnB2015-1, and EC-3-0 presented the lowest severity ratings, and were statistically different from the other genotypes (Table 2). These progenies will be cloned and tested again for Xap and CABMV. Further studies include testing for other pathogens and isolates, as well as field testing for more accurate genotype selection and correlations between disease resistance and plant yield.

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