EFFECT OF *COWPEA APHID-BORNE MOSAIC VIRUS* ON GROWTH AND QUANTITATIVE VARIATION OF TOTAL PHENOLICS AND FLAVONOIDS FROM *PASSIFLORA EDULIS* SIMS

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Abstract - (Effect of *Cowpea aphid-borne mosaic virus* on growth and quantitative variation of total phenolics and flavonoid from *Passiflora edulis* Sims.) *Cowpea aphid-borne mosaic virus* induces woodiness of fruit pericarp, stunting, leaf mosaic and blistering in passion fruit plants. Total amount of phenols and flavonoids from leaves of health and artificially infected plants were quantified by the Folin-Ciocalteu method and reaction with aluminum chloride. Heights of all plants were measured at the beginning and end of the experiment. Infected plants presented 80% less height growth than healthy plants. There was no statistical difference in the amount of total phenols and flavonoids among treatments.

Key words: *Potyvirus*, passion fruit woodiness disease, secondary metabolism.

Resumo - (Efeito do *Cowpea aphid-borne mosaic virus* sobre crescimento e variação quantitativa de fenois totais e flavonoides de *Passiflora edulis* Sims.) *Cowpea aphid-borne mosaic virus* induz o endurecimento do pericarpo do fruto, nanismo, mosaico foliar e bolhas em plantas de maracujá. Os teores totais de fenois e flavonoides de folhas sadias e artificialmente infectadas foram quantificados pelo método de Folin-Ciocalteu e reação com cloreto de alumínio. Alturas de todas as plantas foram medidas no início e no final do experimento. As plantas infectadas apresentaram alturas 80% menores do que as plantas sadias. Não houve diferença estatística nos teores de fenois totais e de flavonoides entre tratamentos.

Palavras-chave: *Potyvirus,* endurecimento dos frutos do maracujazeiro, metabolismo secundário, fenois, flavonoides.

Introduction

Passifloraceae consists of circa 20 genera and 600 species distributed across hot climate regions, such as the Americas and Asia (Souza & Lorenzi 2005). *Passiflora* is the prevailing genus, with approximately 520 species distributed mainly in tropical and subtropical regions, 150 of which are native to Brazil (Cervi 2005). In this country, *Passiflora* species are popularly known as *maracujá*, an indigenous word meaning “fruit to suck to” or “gourd-shaped fruit” (Teixeira 1994). Passion fruit plants (*Passiflora* spp.) are climbing, sub woody ivies that produce grape-shaped fruits (Cunha *et al*. 2004).

Brazil is the world’s largest producer of the yellow passion fruit (*Passiflora edulis* Sims.), with a cultivated area of around 62,019 ha (Agrianual 2013). Within the country, the northeast region has the largest production figures (Meletti 2011). Only two passion fruit species are commercially important, the yellow passion fruit (sometimes called sour passion fruit), which is used in the juice industry, and *P. alata* Curtis (the sweet passion fruit), destined for *in natura* consumption. Known in popular medicine for their pharmacological properties for which their secondary metabolites are responsible, passion fruit varieties are considered as functional foods. These fruit present antioxidant and medicinal properties, and are used in the treatment of anxiety and irritability (Nodari *et al*. 2000, Dhawan *et al*. 2004). Patel (2009) described the antihypertensive and antioxidant action of a *P. edulis* methanolic fraction, which was shown to contain polyphenols, while Zeraik *et al*. (2010) reported that the antioxidant action of these fruit is due to the presence of flavonoids. However, commercial passion fruit cultures are likely to acquire a series of different diseases, mainly caused by viruses (Anjos *et al*. 2001, Chagas & Colariccio 2006, Fisher & Rezende 2008).

Passion fruit woodiness (PFW), the most important disease to affect passion fruit plantations in Brazil, is caused by the *Cowpea aphid-borne mosaic virus* (CABMV, *Potyvirus*). This viral infection causes hardening of the pericarp, a downside that lowers fruit quality, reducing marketable production numbers and leading to economic losses (Peruch *et al*. 2009). Other systemic symptoms such as mosaic and blistering are also associated to PFW (Bock & Conti 1974).

In spite of this, the literature lacks scientific papers describing the influence of CABMV in the metabolism of phenols and flavonoids present in the passion fruit. In this scenario, the present study aimed to assess the role of CABMV in the growth of the yellow passion fruit, and its effect on the levels of total phenols and flavonoids, by comparing extracts obtained from healthy and CABMV-infected leaves.

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Material and methods

The CABMV isolate was obtained from *P. edulis* (yellow passion fruit) produced in Monte Alegre do Sul, state of São Paulo, Brazil, and characterized according to Silva *et al*. (2012). Sample leaves, collected in the field and stored in calcium chloride at -20ºC, were triturated with previously sterilized and chilled mortar and pestle containing a 1:5 (g:mL) sodium sulfite 0.5% medium (pH 6.0) (Gibbs & Harrison 1976) to obtain the viral inoculum. Fifty-four yellow passion fruit plants grown from seeds (IAC-277) sowed in previously sterilized soil and kept in a greenhouse were used in experiments to assess growth and variation in total phenol and flavonoid contents. Ninety days after germination, the plants were randomized into three treatment groups: plants rubbed on with a sodium sulfite 0.5% solution (L1); plants inoculated with CABMV + sodium sulfite 0.5% solution (L2); and a control group (C). Mechanical infection of CABMV onto L2 plants was carried out rubbing the viral inoculum on the adaxial epidermis of the third leaf above the cotyledonary node axil. L1 plants had the same leaves rubbed, but with the buffer solution only. Control plants were not exposed to any treatment. Within each group of 18 plants, three randomized repeats (n = 3) were carried out. The virus was detected by PTA-ELISA using a CABMV-specific antiserum.

Growth was assessed measuring plant height of each individual prior to and after inoculation. Quantification of total phenols and flavonoids was conducted 30 days after inoculation using 2.0 g of dried leaves. Powdered leaves were macerated under heating in MeOH 80% reflux for 1 h. The extraction procedure was repeated three times. Extracts were filtered, pooled, concentrated in a rotatory evaporator, washed in toluene and resuspended in MeOH 100% (according to Furlan *et al*. 2010, with modifications). Total phenols were quantified according to the Folin-Ciocalteu method (Waterman & Mole 1994), while flavonoids were measured by reaction with aluminum chloride (adapted from Motta *et al*. 2005) using *p*-coumaric acid and quercetin as reference, respectively.

Mean contents of total phenols and flavonoids were evaluated in ANOVA and compared using the Tukey test (α = 5%).

Results and discussion

PTA-ELISA confirmed that groups C and L1 plants were healthy, while L2 plants were infected by CABMV. Apart from the positive results obtained in the assay, L2 plants exhibited typical visible symptoms such as mosaic and blistering on leaves (Figure 1). This viral infection influenced growth of L2 plants, whose mean growth values were 80% lower, in comparison with C and L1 (Table 1). Since mean height of plants challenged with buffer (L1) did not differ from the height of control individuals, it is possible to conclude that this decrease in height was indeed caused by CABMV infection.

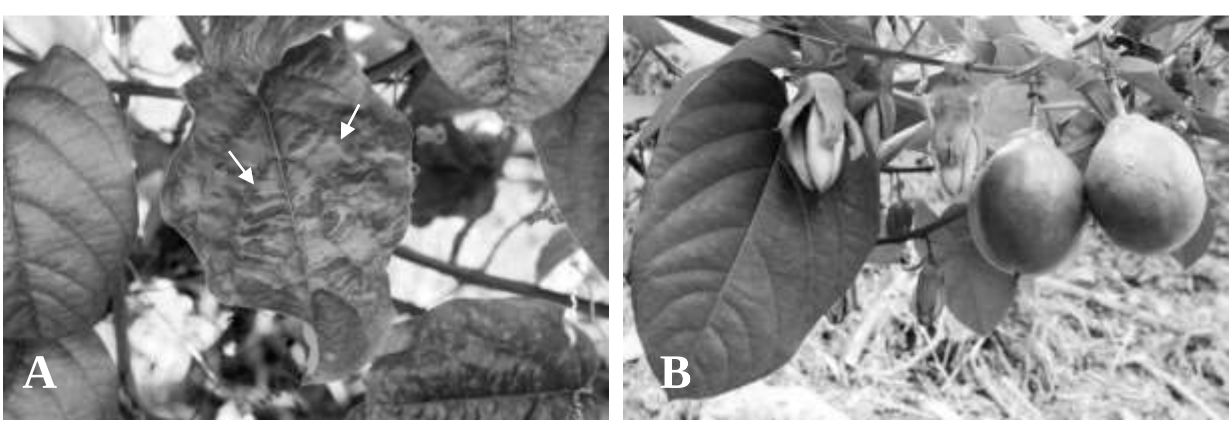

Fig. 1: (A) Mosaic and blistering (arrows) in *Passiflora edulis* Sims. infected with CABMV. (B) Healthy *Passiflora edulis* Sims. (Photo credits: Marcelo Eiras.)

Pathogens like viruses, phytoplasms and nematodes induce symptoms such as chlorosis and dwarfism, and therefore directly affect photosynthesis and development of the host plant (Agrios 2005). These symptoms are seen as phenotypical changes in cell physiology and structure associated with impaired growth and development (Maule *et al*. 2002). Agrios (2005) and Hull (2009) observed that lower growth is the main symptom prompted by viruses in plants, while Fisher & Rezende (2008) added that, apart from CABMV, the *Passion fruit woodiness virus* (PWV, *Potyvirus*) also induces lower growth and development of passion fruit species.

In spite of the marked effect of viral infections on passion fruit development, no statistically significant difference was observed in total phenol and flavonoid contents across treatments in the present study (Table 1).

Table 1: Total phenols and flavonoids levels (µg/mg dry leaf) in methanolic extracts of *Passiflora edulis* Sims.

Treatment	Height[1,4]	Total phenols[2,4]	Total flavonoids[3,4]
C	25.833[a] ± 17.5744	25.233[a] ± 8.708	1.320[b] ± 0.382
L1	24.056[a] ± 15.2937	26.328[a] ± 5.572	1.332[b] ± 0.170
L2	5.3333[b] ± 6.167	33.413[a] ± 7.223	0.992[b] ± 0.169

Obs.: Mean ± SD difference in initial *vs.* final height (cm) of plants challenged with each treatment. C: control; L1: sodium sulfite 0.5%; L2: challenge with CABMV + sodium sulfite 0.5%. 1- cm; 2- µg E *p*-cumaric acid/mg dry leaf; 3- µg E quercetin/mg dry leaf; 4- Values followed by identical letters do not present statistically significant difference (α = 0.5%).

Increased total phenol contents have clearly been shown to be a response produced by plants infected with fungi (Vidhyasekaran 2004, Agrios 2005). The phenolic content, from methanol extratcts of *Passiflora nitida* Kunth and *P. foetida* L. were shown to have antimicrobial activity against *Escherichia coli* by agar diffusion and turbidity assays (Bendini *et al*. 2006). However, the actual influence of a viral infection on phenol levels has yet to be fully investigated. Ajmal *et al*. (2011) analyzed *Gossypium* spp. infected with Cotton leaf curl virus (CLCuV, *Begomovirus*) and observed a decrease in phenol contents of leaves. Similarly, a drop in phenol levels was reported for directly infected (local infection) leaves of *Datura stramonium* L. challenged with *Potato virus X* (PVX, *Potexvirus*) by Duarte *et al*. (2008). However, the authors also observed that levels of these metabolites were higher in leaves challenged only with phosphate buffer and in leaves above the challenged ones, possibly induced by mechanical injury. The results obtained in the present study for the described passion fruit CABMV system do not confirm the premise that mechanical injury and/or viral infection induce some change in total phenol contents in passion fruit plants.

Considering flavonoid levels, few studies have proved the influence of a virus on levels of these metabolites. As the results herein reveal, CABMV infection or mechanical injury *per se* did not affect total flavonoid contents in yellow passion fruit plants (Table 1). Nevertheless, the literature has produced diverse results as to the effect of viruses on levels of this class of compounds. Kreft *et al*. (1999) report a decrease in rutin levels, a well-known flavonoid, in *Solanum tuberosum* L. susceptible to *Potato virus Y* NTN (PVY^{NTN}, *Potyvirus*). However, in an elegant study that shed new light on the effect of the Grapevine leaf-roll-associated virus-3 (GLRaV-3, *Ampelovirus*) on *Vitis vinifera* L. var. Cabernet Sauvignon, Vega *et al*. (2011) found that flavonoid levels first increased, only to drop afterwards, during the ripening period of fruits. Kreft *et al*. (1999) and Vega *et al*. (2011) did not identify any qualitative difference in flavonoids profiles of plants infected with viruses and healthy individuals.

Phenolic compounds such as flavonoids are believed to play a role in a plant's defense against the attack by pathogens (Croteau *et al*. 2000), and their levels might be higher in infected plants, compared with healthy individuals. In spite of the fact that CABMV significantly changes the growth of *P. edulis*, this effect is not observed in secondary metabolism, which involves the action of flavonoids and diverse phenolic substances, as well as other compounds (Agrios 2005, Dewick 2009).

Conclusion

In spite of the fact that CABMV changes the growth of *P. edulis*, leading to a marked decrease in plant height, no statistically significant differences were observe in total phenol and flavonoid levels between healthy and experimentally challenged yellow passion fruit plants.

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