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Salicylic acid: resistance inducer to two-spotted spider mite in strawberry crop

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ABSTRACT

The strawberry is susceptible to several pests which cause a reduction in productivity. The use of mechanisms which repel or prevent these pests to establish, represent a sustainable environmental technology to reduce the frequency of agrochemical use. In this context, the aim of this study was to evaluate the effect of salicylic acid (SA) on resistance induction against two-spotted spider mite in strawberry cultivars (Aromas and Sweet Charlie). Five concentrations of SA (0, 25, 50, 75 and 100 mg L^{-1}) were tested in order to verify the antixenosis effects. In SA concentration of 50 mg L^{-1}, a reduced number of mites in the two strawberry cultivars was noticed. For the number of eggs deposited on leaflet, we verified an effect of SA concentration with quadratic adjustment in the equation. Sweet Charlie cultivar was more effective than Aromas in relation to the reduction of mite oviposition on leaflets treated with SA. SA concentrations of 25, 50 and 75 mg L^{-1} were the most efficient for the evaluated traits allowed to infer its potential as antixenotic resistance inducer against two-spotted spider mite in strawberry crop.

Keywords: *Fragaria x ananassa*, two-spotted spider mite, antixenosis.

RESUMO

Ácido salicílico: indutor de resistência ao ácaro rajado na cultura do morangueiro

O morangueiro é atacado por diversas pragas que ocasionam redução na produtividade. Mecanismos que repelem ou evitam que essas pragas se estabeleçam representam uma tecnologia ambientalmente correta e sustentável, para reduzir a frequência no uso de agroquímicos. Neste contexto, objetivou-se avaliar o efeito do ácido salicílico (AS) na indução de resistência ao ácaro-rajado (*Tetranychus urticae*), nas cultivares de morangueiro Aromas e Sweet Charlie. Foram testadas cinco concentrações de AS (0, 25, 50, 75 e 100 mg L^{-1}) para verificar possíveis efeitos de antixenose. Houve redução da sobrevivência do ácaro na concentração de AS em 50 mg L^{-1} nas duas cultivares de morangueiro. Para o número de ovos depositados sobre o folíolo, verificou-se efeito de concentração de AS com ajuste quadrático da equação. A cultivar Sweet Charlie foi mais efetiva que a Aromas na redução da oviposição dos ácaros sobre os folíolos tratados com AS. Concentrações de 25, 50 e 75 mg L^{-1} de AS foram as mais eficientes para as características avaliadas, permitindo inferir o seu potencial como indutor de resistência tipo antixenose para o ácaro-rajado na cultura do morangueiro.

Palavras-chave: *Fragaria* x *ananassa*, ácaro rajado, antixenose.

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Strawberry crop is highly susceptible to pests, which can cause reduction in productivity, although having a short production cycle. In general, farmers use many preventive agrochemical applications; however, the use of agrochemicals as the only or main pest management tactics can cause severe damage to environment, biological imbalance, rural worker and consumer health damage, and also an increase in production costs and residue accumulation in marketable fruits (Cavalcanti *et al*., 2010).

Among these, two-spotted spider mite (*Tetranychus urticae*) is one of the main pests of strawberry crop in Brazil (Moraes & Flechtmann, 2008; Iwassaki *et al*., 2015), causing mesophilic cell damage and stomata closure, reduction of photosynthetic rate of the plant and reduction in final crop production. Synthetic acaricides are the most used substances to control this pest (López *et al*., 2014; Bernardi *et al*., 2015). The control is difficult to manage since this spider mite has high reproductive potential and products used to kill mites show low efficiency in several populations which are resistant to acaricides abamectin and fenpyroxime (Sato *et al*., 2009).

So, SA acts as a regulator in biological processes in plants, including defense (Kumar *et al*., 2015). It acts on the accumulation of superoxide and hydrogen peroxide in the apoplast, causing cell death at infection site, promoting lignin synthesis in the cell wall, making it difficult stylet penetration and chewing of insects, due to cell wall stiffening (Datnoff *et al*., 1991; Epstein, 1994; Marschner, 1995), acting in the establishment of systemic acquired resistance (Gao *et al*., 2015). Thus, stimuli which repel or prevent pests in crops of agronomic interest, have broad potential to reduce the frequency and use of agrochemicals in fields.

Plant volatile emissions can be induced by the exogenous application of plant hormones such as jasmonic acid and salicylic acid (Tholl *et al*., 2011); however, the role of SA in the production and release of volatiles from pest-repelling plants is still poorly known. Therefore, this study aims to contribute to better understanding the role of this elicitor in the repellency of plants against spider mite attack on strawberry, evaluating concentrations of SA as inducer of resistance to *T. urticae* in two strawberry cultivars.

MATERIAL AND METHODS

Two bioassays were carried out under heated greenhouse conditions, at temperature ranging from 22ºC to 25ºC. The authors used strawberry cultivars Aromas and Sweet Charlie, grown in 3 dm^3-capacity pots with mixture of sifted soil and Tropstrato substrate, using 20 g fertilizer formula 4-14-08. Every 15 days, top-dressing fertilization was performed, intercalating two fertilizers: one formulated 12-06-12 (10 g) and one soluble 15-15-20 (100 mL ha^{-1}) (Embrapa, 2011). The pots were kept in a greenhouse with micro-sprinkler irrigation performed daily, during plant development. Routine cultural practices for strawberry crop were done, except for phytosanitary management using chemical defenses.

Thirty days after transplant, the authors started leaf applications using AS (diluted with distilled water) on plants up to runoff point, using concentrations of 0, 25, 50, 75 and 100 mg L^{-1}, with the aid of manual sprayer. All over the leaf surface was sprayed, with a seven-day interval between applications, totalizing 10 applications up to starting bioassays with two-spotted spider mite. Treatments with SA were spaced 3 m in the greenhouse in order to avoid interference among each other, throughout the whole experiment.

The mites used were provided by Departamento de Entomologia e Acarologia from Escola Superior de Agricultura "Luiz de Queiroz".

In the free-choice bioassay, the authors evaluated the two-spotted spider mite preference in relation to the two strawberry cultivars with different SA concentrations. Arenas consisting of Petri dishes (60 mm diameter), composed of a layer of cotton, superimposed on a sponge saturated with water were used. In the opposite sides, strawberry leaf discs were placed (3 cm diameter) with two treatments in each disc (control and each one of the other treatments) using rigid transparent PVC plastic sheets, which were cut (18 x 18 mm) and then connected by a plastic cover (18 x 18 mm), being discarded after use.

Leaf discs were obtained from central part of leaflets, collected from medium leaves of strawberry plants at 80 days of development, previously washed with distilled water, being placed on Petri dishes, abaxial surface facing up in the arena. With the aid of a fine brush and stereomicroscope (Nikon 1510), six adult two-spotted spider mites were released in the center of each dish, thus allowing free passage and access of the mites to the leaflets of both sides.

The dishes were properly closed and kept in BOD type climatized chambers, at 25±1ºC temperature, 70±10% U.R. and 12 h photophase. Mites were counted within 24 hours, being observed every hour in this time interval. The experiment was carried out in a completely randomized design, in a factorial scheme 2x5, with two strawberry cultivars (Aromas and Sweet Charlie) and five SA concentrations (0, 25, 50, 75, 100 mg L^{-1}), with 10 replicates. The cultivars used in this experiment are those which best fitted to the evaluated cultivation region.

In the non-choice bioassay, the authors used arenas with a strawberry leaf disc in each of them (3 cm diameter) of each treatment, abaxial surface facing up. Onto each disc, six female adults were transferred, with the aid of a fine brush and stereomicroscope (Olympus SZ51). The females were kept on the discs for 24 hours, and then number of mites and number of deposited eggs (NTOD) on each leaf disc were counted. We used completely randomized experimental design, in a factorial scheme 2x5, using two strawberry cultivars (Aromas and Sweet Charlie) and five SA concentrations (0, 25, 50, 75 and 100 mg L^{-1}), with 10 replicates. The dishes remained closed in different BOD type climatized chambers at temperature of 25±1ºC, 70±10% U.R. and 12 h photophase.

After verifying normality and homogeneity using Shapiro-Wilk and Bartlett tests, respectively, data were submitted to computer statistical software SISVAR and variance analysis was done; then, averages were compared through Tukey test at 5% probability.

For free-choice antixenosis data, the trend using percentages of choice were calculated using graphics created by software Microsoft Excel (2016). The equations were derived to determine the point of maximum concentration applied with better resistance induction efficiency. Non-choice and free-choice antixenosis tests were transformed by equation $(x + 0.5)^{1/2}$ Pearson correlations were estimated comparing SA concentrations with sources of variation. Significance of estimated correlation values and R^2 were obtained through *t* test at 5%.

RESULTS AND DISCUSSION

Free-choice trial

In this test, the authors verified that the two-spotted spider mite showed a preference for moving towards the strawberry cultivars without SA application (Figure 1), showing antixenosis type resistance (non-preference). Concentrations of 25, 50 and 75 mg L^{-1} were the most effective in repelling the two-spotted spider mite movement (Table 1). This effect can be attributed to the resistance mechanism which manifests so that the plant survives the attack by herbivores, considering this defense mechanism called Systemic Acquired Resistance (SAR) (Van Loon *et al*., 1998). SAR is a result of identification of invader, accompanied by the induction of the synthesis of specific substances, such as chitinases and other hydrolytic enzymes, due to elicitor action, which acts as an endogenous signal to trigger the plant

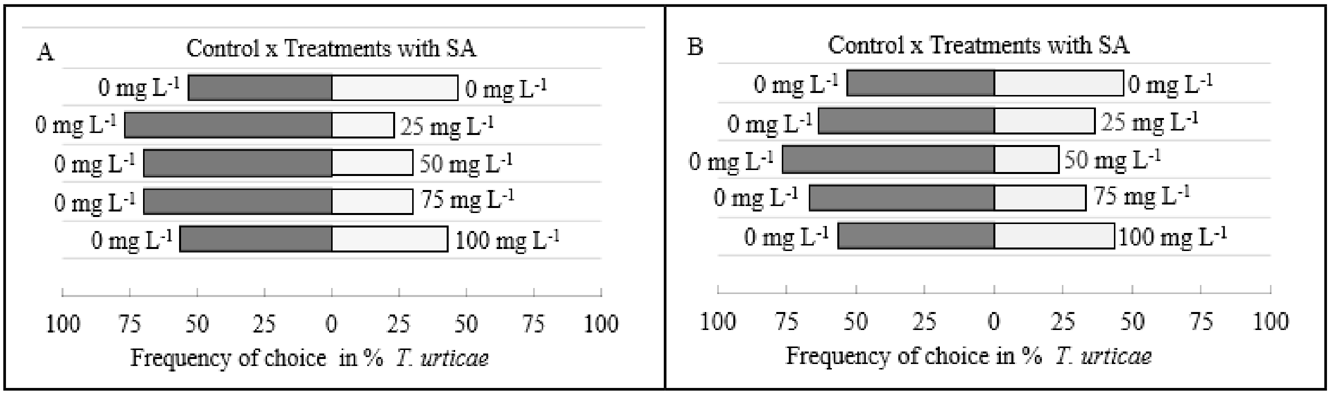

Figure 1. Frequency of choice of *Tetranychus urticae* on foliar discs of strawberry cultivars. A) Aromas and B) Sweet Charlie, with salicylic acid (SA) application at concentrations of 0, 25, 50, 75, 100 mg L^{-1} using a free-choice test. Temperature of 25±1ºC, 70±10% U.R. and 12 h photophase. Guarapuava, UNICENTRO, 2017.

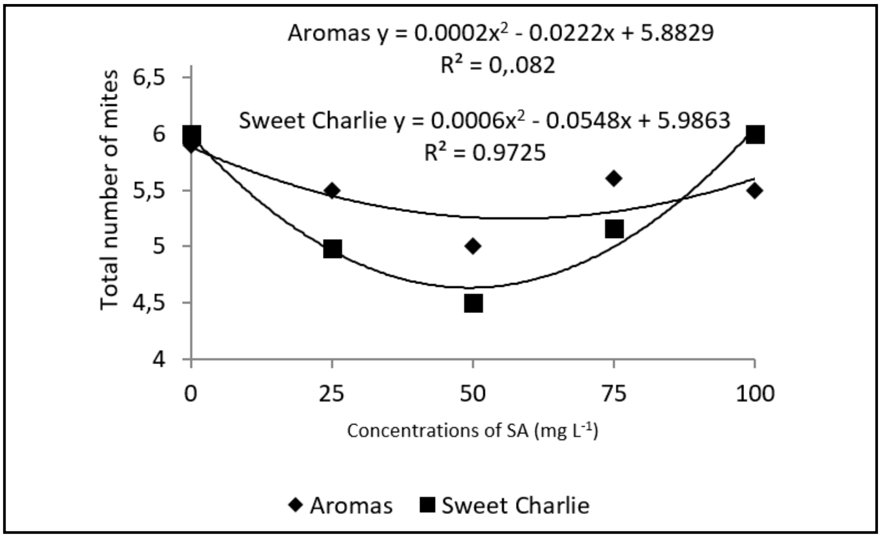

Figure 2. Total number of adult mite *Tetranychus urticae* (NTA) on leaf discs of strawberry cultivars Aromas and Sweet Charlie in relation to SA Aromas and Sweet Charlie concentrations, 0, 25, 50, 75, 100 mg L^{-1}, in a free-choice test. Temperature of 25±1ºC, 70±10% U.R. and 12 h photophase. Guarapuava, UNICENTRO, 2017.

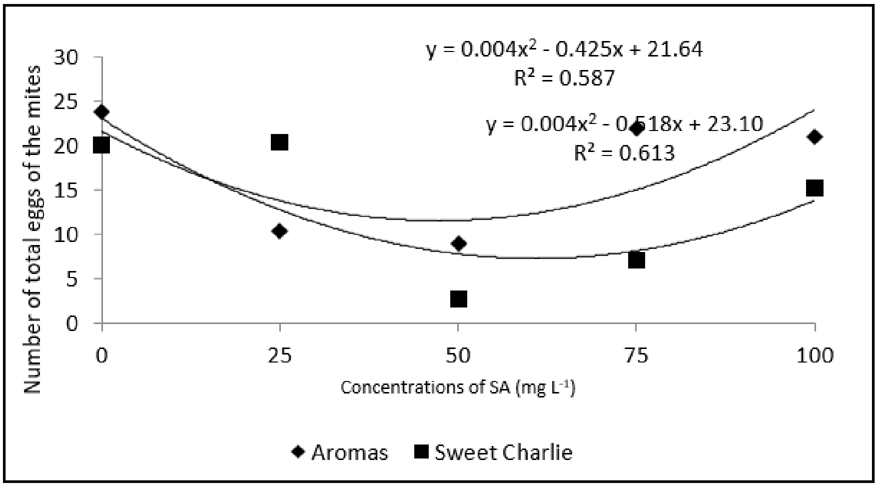

Figure 3. Number of total deposited eggs of *Tetranychus urticae* (NTOD) on surface of leaf discs of strawberry cultivars Aromas and Sweet Charlie in relation to salicylic acid (SA) concentrations, 0, 25, 50, 75, 100 mg L^{-1}, in a non-choice test. Temperature of 25±1ºC, 70±10% U.R. and 12 h photophase. Guarapuava, UNICENTRO, 2017.

defense response (Mandal *et al*., 2008).

The authors noticed that the lowest AS doses were enough to repel the two-spotted spider mite from strawberry plants (Figure 1), however, the highest AS dose (100 mg L^{-1}) did not show effect on repellency, showing similar behavior when comparing to the control (Table 1). According to DelRio *et al*. (2006), high AS concentration promote higher production of oxygen-reactive species (EROs), resulting in an antagonistic effect on plant protection.This fact could explain negative effects of high AS concentration on behavior of the mite found in this study: instead of protecting the attacked parts, EROs could have accelerated tissue degradation, leaving the plant even more vulnerable to mite attack. If stress is more severe, it considerably increases the production of free radicals which can lead to a cascade of events starting with lipid peroxidation, advancing towards membrane degradation and cell death (Greggains *et al*., 2000).

Non-choice trial

In this bioassay, the authors verified a significant interaction between cultivar and SA concentration for total number of adult mites (NTA) and for total number of deposited eggs (NTOD) (Figures 2 and 3), respectively.

For NTA, on leaflet surface, quadratic adjustment in the equations for applied concentrations in strawberry cultivars (Figure 2) was noticed. In SA intermediate concentrations (55.50 mg L^{-1} and 45.66 mg L^{-1}), the authors found the maximum reduction in number of

Table 1. Attractiveness (number of attracted *Tetranichus urticae* in free-choice test), total number of adult mites which survived (NTA) and total number of eggs deposited (NTOD) on strawberry cultivars Aromas and Sweet Charlie, submitted to SA application (0, 25, 50, 75, 100 mg L^{-1}). Guarapuava, UNICENTRO, 2017.

Cultivar	Dose of SA (mg L^{-1})					
	0	25	50	75	100	Average
Aromas	3.2 aA	1.4 aB	1.6 aB	1.8 aB	2.8 aA	2.1 a
Sweet Charlie	3.2 aA	1.6 aB	1.4 aB	2.0 aB	2.8 aA	2.2 a
Average	3.2 A	1.5 B	1.6 B	1.9 B	2.8 A	
CV (%)	7.31					
	NTA					
Aromas	5.9 aA	5.5 aA	5.0 aC	5.5aA	5.5 aA	5.5 a
Sweet Charlie	5.9 aA	5.4 a B	5.2 aC	5.3aB	5.3 aB	5.4 a
Average	5.9 A	5.4 AB	5.1 C	5.4 AB	5.4 AB	
CV (%)	15.04					
	NTOD					
Aromas	23.8 aA	10.4 aB	9.0 aC	21.1aB	22aA	17.2 a
Sweet Charlie	22.1 bA	20.5 bB	2.8 bC	7.2 bB	15.2bB	13.1 b
Average	22.9 A	15.4 B	5.9 C	14.1 B	18.6 AB	
CV (%)	42.41					

Averages followed by same uppercase letter in line and lowercase letter in column do not differ significantly from each other by Tukey test at 5% ($p < 0.05$).

mites for cultivars Aromas and Sweet Charlie, respectively. In average test, the dose of 50 mg L^{-1}, showed the best response, it means that it shows the lowest number of live mites, with significant difference in relation to other doses was verified (Table 1).

For NTOD on the leaflet, similar effect of SA on NTA with quadratic adjustment in the equation was noticed (Figure 3). The points of minimum oviposition were estimated in 47.27 mg $L^{-1}$and 67.63 mg L^{-1} for cultivars Aromas and Sweet Charlie, respectively (Figure 3). Using average test, the authors verified that the dose of 50 mg L^{-1} was the one which showed the highest effectiveness (Table 1). Similar effect was observed by Shi & Zhu (2013) in exogenous SA application in tomato crop, with a reduction in fecundity and lower survival rates in *Bemisia tabaci*. Donovan *et al*. (2013) also verified similar results with artificial diets and various concentrations of SA in the survival of aphid *Myzus persicae*.

This behavioral biology of the two-spotted spider mite can occur due to responses based on interaction among SA concentrations, genotype and pest. SA acts in the response of the plants after pest attack, altering the activity and increasing the expression of plant defense genes, boosting the response (Ponstein *et al*., 1994). In this study, SA application may have activated strawberry plant defense genes during previous mite attack, and this might be an explanation for the lowest value observed in NTA and NTOD. The results obtained in this study proved that in the study carried out by Silva *et al*. (2005), pests have difficulties to deposit eggs on silicified cells, decreasing the herbivory of the immature stages of these arthropods.

In the last decades, a constant pressure of society to increase the sustainability of agricultural activities could be noticed. This practice requires the creation of innovations and technologies which are less aggressive to the environment and to human health. In this context, using SA is an interesting alternative in order to repel the two-spotted spider mite from strawberry plants, reducing pesticide use in agriculture and, consequently, a reduction in production costs.

SA was an efficient inducer of resistance against the two-spotted spider mite, reducing adult survival and oviposition on leaflets of strawberry cultivars, Aromas and Sweet Charlie, characterizing antixenosis resistance induction.

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