Application of Foliar Synthetic Elicitors for the Management of *Diaphorina citri* (Hemiptera: Liviidae) Populations in Tahiti Lime (*Citrus latifolia* Tanaka)

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Additional index words. brassinosteroids, chitosan, integrated management, proline, salicylic acid, thiamine

Abstract. The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is the most serious threat to the global citrus industry, and its management has depended mainly on the application of chemical insecticides. The application of plant elicitors can contribute to the insect management and also enhance plant physiology. A set of three different experiments was carried out to evaluate the efficacy of foliar applications of synthetic elicitors [salicylic acid (SA), brassinosteroids (Br), chitosan, or thiamine] on the population dynamics of *D. citri* and their effects on the physiology of Tahiti lime trees. The treatments were as follows: trees were sprayed separately with thiamine, SA, or chitosan at a dose of 100 ppm, respectively; trees were treated with foliar brassinosteroid applications at a dose of 1 ppm; and untreated trees (control). By the end of the experiment, the elicitors reduced (75%) the number of adult psyllids compared with the control, where trees treated with chitosan had ≈ 0.5 adult individuals accumulated per flush shoot, whereas the control showed around two individuals. Salicylic acid and thiamine also reduced the number of nymphs compared with the control in the 4 weeks after treatment (WAT) (5.5 vs. 10.08 nymphs, respectively). Treatment with synthetic elicitors also caused a 30% reduction in oviposition by *D. citri*. The foliar applications with Br promoted a greater relative growth rate (RGR) ($44 \text{ mm} \cdot \text{cm}^{-1} \cdot \text{d}^{-1}$) compared with the control treatment and chitosan (24 and $26 \text{ mm} \cdot \text{cm}^{-1} \cdot \text{d}^{-1}$, respectively). Chitosan sprays favored proline synthesis in both flush shoots and leaves. These results suggest that the use of synthetic elicitors can be considered as a tool to reduce the number of applications of chemical insecticides and decrease the development of resistances by *D. citri* because these synthetic elicitors showed an efficacy between 40% and 60% in all its stages in field conditions.

The ACP, *D. citri* Kuwayama (Hemiptera: Liviidae), is currently the most important pest for the global citrus industry because it is the vector responsible for transmitting the bacterial pathogen *Candidatus Liberibacter asiaticus* and *Ca. L. americanus*, the causative agents of huanglongbing disease (HLB) (Halbert et al., 2000). *Diaphorina citri* was officially reported in Colombia in 2007 (Santivañez et al., 2013). However, in 2015, the Colombian Agricultural Institute declared a state of phytosanitary emergency because of the presence of *D. citri* adults infected with the bacteria causing HLB disease (ICA, 2015). The production of acid limes (*Citrus latifolia* Tanaka) or lemons (*Citrus × limon*) is increasing in Colombia because of its export potential, with a

production of 360.836 tons in 2014 (Agronet, 2017). Consequently, the design of long-term strategies to manage the vector insect through integrated pest management (IPM) programs in Colombia is critical to control the spread of HLB disease.

The life cycle of the ACP is closely related to the presence of new flush shoots in plants. In the tropics, the continuous production of new flush shoots allows ACP populations to remain relatively high at various times of the year, rendering control with systemic or contact insecticides difficult because the new growth cannot be continuously protected by pesticides (Pluke et al., 2008; Tsagkarakis et al., 2012). The strategy of applications of synthetic elicitors can be considered as an effective option in arthropod pest management programs because synthetic elicitors are small molecules that can induce immune responses in plants and produce a positive effect in the management of ACP populations (Pawlowski et al., 2016).

Elicitors are compounds that stimulate any type of defense in the plant and favor the synthesis of secondary metabolites under conditions of both abiotic and biotic stress and can be applied through foliar sprays. Within these compounds, carbohydrates, lipids, (glyco)peptides, (glyco)proteins, vitamins, and phytohormones can be found (Angelova et al., 2006; Boubakri et al., 2016; Smith et al., 2009; Thakur and Sohal, 2013; Tsagkarakis et al., 2012). Among the compounds studied to activate defense mechanisms in plants against the attack of biotic agents (macro- and microorganisms) are SA, phytohormones, chitosan, and thiamine (vitamin B1) (Ahn et al., 2005; El Hadrami et al., 2010; Thakur and Sohal, 2013).

Thiamine is a B-complex vitamin that participates in various physiological processes such as glycolysis, the pentose phosphate pathway, and the synthesis of nucleic acids in plants (Goyer, 2010). Thiamine has been used to mitigate aphid infestations in barley and peas (Hamada and Jonsson, 2013). On the other hand, chitosan is a biopolymer that induces defense responses such as lignification, cytoplasmic acidification, phytoalexin biosynthesis, generation of reactive oxygen species, and jasmonic acid biosynthesis in both mono and dicotyledonous plants under conditions of biotic stress (El Hadrami et al., 2010). It has been reported that chitosan has shown insecticidal activity on *Spodoptera littoralis* (Boisd.) and *Aphis nerii* Boyer de Fonscolombe in cotton (Badawy and El-Aswad, 2012).

Phytohormones such as SA and Br also play an important role in signaling to activate resistance against arthropods (Erb et al., 2012). In this regard, it has been widely reported that SA can induce resistance against pathogens, arthropods, and parasitic plants (Smith et al., 2009). Peng et al. (2004) reported that the exogenous application of SA favored the defense response of tomato plants against *Helicoverpa armigera* (Hübner). However, it has been reported that Br can have an antiecdysteroid effect on insects (Richter and Koolman, 1991). Also, Campos et al. (2009) found that Br are an important hormone for the defense against herbivory in the tomato. Still, little is known about the role of Br as inducers of resistance under conditions of herbivory stress and nothing has been reported about the use of this phytohormone as a biorational insecticide for the management of ACP populations.

The expression of physiological attributes of a plant under herbivory conditions is important to characterize defense strategies and to know the potential utility of these expressions as tools for phenotyping or for evaluation of control strategies (Mitchell et al., 2016). Physiological characteristics such as leaf gaseous exchange properties, chlorophylls, leaf temperature, and growth have been useful in identifying plant tolerance against pests (Mitchell et al., 2016). In addition, proline and malondialdehyde (MDA) synthesis plays an important role to characterize plant response to herbivory in

Received for publication 16 Mar. 2018. Accepted for publication 1 May 2018.

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the response of a plant to abiotic and biotic stresses (Rehman et al., 2016; Wei et al., 2007).

Studies on the use of synthetic elicitors have focused mainly on their role in the

control of HLB (Canales et al., 2016; Li et al., 2016). However, literature on the use and efficacy of defense activators or growth regulators for population management of the

ACP remains limited (Tiwari et al., 2013; Tsagkarakis et al., 2012). Therefore, the objective of the present study was to evaluate the biological efficacy of four different synthetic elicitors (SA, Br, chitosan, and thiamine) on ACP individuals (adults, nymphs, and eggs) under field conditions and also to quantify the effect of these treatments on the physiology and nutritional status of Tahiti lime trees.

Materials and Methods

General conditions of the experiments.

Three separate experiments were carried out on the farms 'Parcela 3' (lat. 4°34'09.1"N, long. 74°41'39.3"W, altitude: 313 masl) between June and July 2017, 'Rochela' (lat. 4°33'22.1"N, long. 74°43'36.8"W, altitude: 305 masl) between July and Aug. 2017, and 'Rochelita' (lat. 4°33'07.6"N, long. 74°44'45.6"W, altitude: 263 masl) between Aug. and Sept. 2017. All farms used were irrigated and located in the municipality of Jerusalem in the Department of Cundinamarca in Colombia. The climatic conditions (precipitation and average temperature) during the experiments are presented in Fig. 1. The experiments were conducted on 6, 4, and 2-year-old 'Tahiti lime' (*C. latifolia* Tanaka) grafted on 'Citrumelo CPB 4475' [*Poncirus trifoliata* (L) × *Citrus paradisi* Macfadyen] trees, in the three farms, respectively. Also, all trees were spaced 8 × 5 m.

The treatments were as follows: 1) untreated trees (control), 2) trees treated with foliar thiamine applications at 100 ppm, 3) trees treated with foliar SA sprays at 100 ppm, 4) trees treated with foliar applications of chitosan at 100 ppm, and 5) trees treated with foliar Br applications at 1 ppm of the active ingredient. The concentrations used were chosen based on literature about the effects of these products on the activation of defense mechanisms of the plant (Boubakri et al., 2016; Erb et al., 2012; Pichyangkura and Chadchawan, 2015; Smith et al., 2009). Finally, Table 1 summarizes the characteristics and biological activity of the activators used in this study.

In general, foliar applications of the activators were performed at 0 and 2 weeks after starting the treatments between 0700 and 0900 HR, using a back pump with an application volume of 2 L H₂O per tree, wetting the upper and lower surfaces of the leaves in each test. The chitosan treatment was previously

Fig. 1. Records of precipitation and average temperature for the evaluation trials of synthetic elicitors on the control of populations of *Diaphorina citri* placed in the municipality of Jerusalem (A and B). Data were obtained from the nearest weather station [Jerusalem (lat. 4°56'18'.06"N, long. 74°70'23'.99"W, altitude: 297 masl)] of the Institute of Hydrology, Meteorology, and Environmental Studies of Colombia (IDEAM).

Table 1. Chemical name of the active ingredient, commercial name, and manufacturer of the synthetic elicitors used to evaluate the applications in Tahiti lime trees on populations of *Diaphorina citri*.

Chemical name of active ingredient	Commercial name (manufacturer)	Biological activity
2-Hydroxybenzoic acid	Salicylic acid (Panreac Applichem, Barcelona, España)	Key regulator of biotic agent-induced systemic acquired resistance (SAR)
Thiamine hydrochloride	Thiamine (Sigma Aldrich, St. Louis, MO)	Induces SAR in plants through the salicylic acid and Ca ²⁺ -related signaling pathways
(25 R)-3β. 5α-dihydroxyspirostan-6-one	Biomec DI-31 (Minerales exclusivos SA, Bogotá, Colombia)	Regulate plant development by modulating diverse signaling pathways
2-Amino-2-deoxy-(1→4)-β-D-glucopyranan, poly-(1,4-β-D-glucopyranosamine)	Chitosan from crab shells (Sigma Aldrich)	Favors the biosynthesis of jasmonates that induce the expression of defense response genes

Table 2. Summary of analysis of variance of accumulated individuals of *Diaphorina citri* (adults, nymphs, and eggs) in Tahiti lime trees with and without applications of synthetic elicitors.

	0 ²	1	2	3	4
	WAT				
	Adults				
Farms (F)	$F = 1.88; df = 2, 175; P = 0.155$	$F = 0.66; df = 2, 175; P = 0.521$	$F = 1.79; df = 2, 175; P = 0.170$	$F = 1.28; df = 2, 175; P = 0.281$	$F = 3.15; df = 2, 175; P = 0.045$
Treatments (T)	$F = 0.25; df = 4, 175; P = 0.908$	$F = 2.25; df = 4, 175; P = 0.066$	$F = 2.89; df = 4, 175; P = 0.024$	$F = 4.92; df = 4, 175; P = 0.000$	$F = 6.11; df = 4, 175; P = 0.000$
F × T	$F = 0.55; df = 8, 175; P = 0.814$	$F = 1.10; df = 8, 175; P = 0.363$	$F = 1.18; df = 8, 175; P = 0.316$	$F = 0.77; df = 8, 175; P = 0.633$	$F = 0.96; df = 8, 175; P = 0.467$
	Nymphs				
Farms (F)	$F = 20.09; df = 2, 175; P = 0.000$	$F = 6.67; df = 2, 175; P = 0.001$	$F = 5.58; df = 2, 175; P = 0.004$	$F = 7.32; df = 2, 175; P = 0.000$	$F = 10.21; df = 2, 175; P = 0.000$
Treatments (T)	$F = 1.76; df = 4, 175; P = 0.139$	$F = 1.61; df = 4, 175; P = 0.175$	$F = 3.12; df = 4, 175; P = 0.017$	$F = 4.09; df = 4, 175; P = 0.003$	$F = 5.64; df = 4, 175; P = 0.000$
F × T	$F = 2.59; df = 8, 175; P = 0.011$	$F = 1.46; df = 8, 175; P = 0.177$	$F = 1.35; df = 8, 175; P = 0.222$	$F = 1.57; df = 8, 175; P = 0.136$	$F = 1.74; df = 8, 175; P = 0.093$
	Eggs				
Farms (F)	$F = 10.84; df = 2, 175; P = 0.000$	$F = 5.93; df = 2, 175; P = 0.003$	$F = 12.45; df = 2, 175; P = 0.000$	$F = 18.87; df = 2, 175; P = 0.000$	$F = 19.84; df = 2, 175; P = 0.000$
Treatments (T)	$F = 1.69; df = 4, 175; P = 0.145$	$F = 6.17; df = 4, 175; P = 0.000$	$F = 8.34; df = 4, 175; P = 0.000$	$F = 8.88; df = 4, 175; P = 0.000$	$F = 11.28; df = 4, 175; P = 0.000$
F × T	$F = 1.54; df = 8, 175; P = 0.146$	$F = 1.17; df = 8, 175; P = 0.320$	$F = 1.62; df = 8, 175; P = 0.124$	$F = 1.91; df = 8, 175; P = 0.061$	$F = 2.53; df = 8, 175; P = 0.012$

²Foliar applications of synthetic elicitors were performed at 0 and 2 WAT started.

WAT = weeks after treatments.

dissolved in acetic acid at a concentration of 1% to facilitate foliar application. All foliar applications did not include an adjuvant. The experimental unit (treatment) consisted of three trees in each farm that were used in their respective experiment. In addition, the treatments of synthetic elicitors were arranged in a randomized complete block design where

each treatment was repeated four times (four blocks). Therefore, a total of 12 trees were used per treatment in each farm per resistance activator test. In all treatments, guard trees were used to avoid edge effects due to drift by foliar elicitor sprays.

Population dynamics of ACP individuals.
The methodology described by Sétamou et al.

Fig. 2. Average accumulated individuals of *Diaphorina citri* for adults (A), nymphs (B), and eggs (C) of three different experiments developed in Tahiti lime trees treated with and without synthetic elicitors. Arrows indicate foliar synthetic elicitor applications.

(2008) was used to estimate the population variability of *D. citri*. Relative infestation levels of adults, eggs, and nymphs of *D. citri* were estimated by randomly reviewing five young flush shoots (0.5–1.0 cm) per tree at each of the four cardinal points. In addition, eggs and nymphs were counted in situ and registered per flush shoot using a 15X hand-held lens. In general, samples for both tests were collected between 0 and 4 WAT at intervals of 7 d per sampling.

Stomatal conductance (g_s) and leaf temperature. The g_s was determined in fully expanded leaves from the middle part of the terminal shoot of the season's growth using a porometer (SC-1; Decagon Devices, Pullman, WA). The g_s measurements were carried out between 0900 and 1400 HR, taking a leaf at two cardinal points (west and east) in each experiment. Then, leaf temperature was determined using an IR thermometer at noon (Model 39755-10; Cole Parmer, Vernon Hills, IL) comprising the average of two measurements per tree. Finally, the readings were performed at 2 and 4 WAT.

Efficiency of photosystem II (F_v/F_m) and relative chlorophyll content. A continuous excitation fluorometer (Handy PEA; Hansatech Instruments, Kings Lynn, UK) was used to estimate the F_v/F_m ratio. The readings were determined with a maximum pulse of light intensity of up to $2.600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on the surface of the leaf sample, which was previously adapted to the dark over a 10-min period. Subsequently, the relative chlorophyll content was estimated by a chlorophyllometer (Chl Plus; atLEAF, Wilmington, DE). Measurements of F_v/F_m and relative chlorophyll content were also taken at 2 and 4 WAT on leaves located at two cardinal points of the tree canopy (west and east).

Relative growth rate. Four young terminal flush shoots (≤ 0.5 cm) were marked at the cardinal points of each tree. Then, the length of each flush shoot was recorded at 2 and 4 WAT. The relative rate of the flush shoots in each treatment was estimated by the following equation (García-Castro et al., 2017):

$$\text{RGM} = \frac{\ln(\text{flush shoot length time2}) - \ln(\text{flush shoot length time1})}{\text{time2} - \text{time1}}$$

Malondialdehyde content in flush shoots and leaves of Tahiti lime trees. The thiobarbituric acid method described by Hodges et al. (1999) was used for the determination of lipid peroxidation MDA at 4 WAT. About 0.3 g of plant material from terminal flush shoots of the season's growth (≤ 5 cm) and mature leaves were homogenized and stored with liquid nitrogen for analysis. After centrifugation, the absorbances at 440, 532, and 600 nm were estimated with a spectrophotometer (Spectronic BioMate 3 ultraviolet-Vis; Thermo, Madison, WI). Finally, an extinction coefficient ($157 \text{ M}\cdot\text{mL}^{-1}$) was used to obtain the MDA concentration.

Proline content in flush shoots and leaves of Tahiti lime trees. The method described by Bates et al. (1973) was used to estimate the

Table 3. Effect of foliar applications of synthetic elicitors on stomatal conductance (g_s), leaf temperature, chlorophyll content, and PSII efficiency in Tahiti lime trees.

	g_s ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		Leaf temperature ($^{\circ}\text{C}$)		Chlorophyll content (Atleaf units)		Efficiency PSII (F_v/F_m)	
	2 ^a	4	2	4	2	4	2	4
Control	257.53 $\pm$ 42.95 ^y	125.30 $\pm$ 19.39 a	36.44 $\pm$ 0.66 a	34.63 $\pm$ 1.53 a	65.58 $\pm$ 1.23 a	65.54 $\pm$ 0.94 a	0.72 $\pm$ 0.03 a ^x	0.74 $\pm$ 0.03 a
Thiamine	288.25 $\pm$ 33.63 a	153.03 $\pm$ 17.58 a	35.99 $\pm$ 0.99 a	35.43 $\pm$ 0.69 a	63.54 $\pm$ 1.28 a	64.12 $\pm$ 1.38 a	0.77 $\pm$ 0.01 a	0.78 $\pm$ 0.01 a
Salicylic acid	289.82 $\pm$ 28.16 a	166.50 $\pm$ 27.77 a	35.90 $\pm$ 1.03 a	34.24 $\pm$ 0.67 a	64.46 $\pm$ 0.88 a	64.95 $\pm$ 0.93 a	0.76 $\pm$ 0.01 a	0.76 $\pm$ 0.01 a
Chitosan	246.76 $\pm$ 49.45 a	130.21 $\pm$ 18.07 a	36.56 $\pm$ 0.59 a	37.10 $\pm$ 0.88 a	65.77 $\pm$ 0.86 a	63.77 $\pm$ 1.56 a	0.74 $\pm$ 0.01 a	0.76 $\pm$ 0.01 a
Brassinosteroids	287.33 $\pm$ 46.98 a	136.59 $\pm$ 10.90 a	36.36 $\pm$ 0.96 a	35.52 $\pm$ 0.93 a	66.03 $\pm$ 0.68 a	65.68 $\pm$ 1.01 a	0.74 $\pm$ 0.01 a	0.78 $\pm$ 0.01 a
Farms (F)	$F = 23.93$; $df = 2,59$; $P = 0.000$	$F = 29.56$; $df = 2,59$; $P = 0.000$	$F = 8.97$; $df = 2,59$; $P = 0.000$	$F = 11.60$; $df = 2,59$; $P = 0.000$	$F = 1.31$; $df = 2,59$; $P = 0.280$	$F = 2.14$; $df = 2,59$; $P = 0.129$	$F = 8.51$; $df = 2,59$; $P = 0.000$	$F = 1.36$; $df = 2,59$; $P = 0.268$
Treatments (T)	$F = 0.44$; $df = 4,59$; $P = 0.782$	$F = 1.69$; $df = 4,59$; $P = 0.171$	$F = 0.10$; $df = 4,59$; $P = 0.981$	$F = 2.00$; $df = 4,59$; $P = 0.111$	$F = 1.07$; $df = 4,59$; $P = 0.382$	$F = 0.43$; $df = 4,59$; $P = 0.782$	$F = 1.60$; $df = 4,59$; $P = 0.193$	$F = 1.18$; $df = 4,59$; $P = 0.333$
F $\times$ T	$F = 0.52$; $df = 8,59$; $P = 0.832$	$F = 1.13$; $df = 8,59$; $P = 0.365$	$F = 0.62$; $df = 8,59$; $P = 0.759$	$F = 2.62$; $df = 8,59$; $P = 0.020$	$F = 1.20$; $df = 8,59$; $P = 0.323$	$F = 1.51$; $df = 8,59$; $P = 0.183$	$F = 1.69$; $df = 8,59$; $P = 0.129$	$F = 0.81$; $df = 8,59$; $P = 0.601$

^aReadings were carried out at 0 and 2 weeks after the treatments started.

^yEach datum represents the mean of four values $\pm$ SE.

^xDifferent letters are significantly different, according to Tukey's test at $P \leq 0.05$.

proline content. Furthermore, 0.3 g of plant material collected from flush shoots and mature leaves was homogenized and stored with liquid nitrogen at 4 WAT. Absorbance was measured at 520 nm using a spectrophotometer (Spectronic BioMate 3 ultraviolet-Vis, Thermo). The proline content was determined by a standard curve and was calculated in fresh weight based on the following formula:

$$\text{Proline content} = \frac{\mu\text{mol Proline}}{\text{g fresh plant material}} \left[\frac{\left(\frac{\mu\text{g Proline}}{\text{mL}} \times \text{mL Toluene} \right)}{\frac{115.5 \mu\text{g}}{\mu\text{mol}}} \right] = \frac{\left[\frac{\text{g sample}}{5} \right]}{\left[\frac{\mu\text{mol}}{\text{g sample}} \right]}$$

Plant tissue analysis. Leaf and flush shoots samples were only collected at the end of the third experiment (Farm 'la Rochelita') for the purpose of estimating whether the foliar application of synthetic elicitors could affect the nutritional status of Tahiti lime trees. Forty mature leaves and 20 young terminal flush shoots (≤ 0.5 cm) were separately collected

Fig. 4. Effect of foliar applications of synthetic elicitors on the content of malondialdehyde (MDA) and proline in flush shoots (A and B) and leaves (C and D) of Tahiti lime trees. NS = not significant at $P \leq 0.05$. Columns with different letters indicate differences according to the Tukey test ($P \leq 0.05$). Each bar represents the average of four blocks $\pm$ SE.

Table 4. Concentration of macro- and micronutrients in leaves of Tahiti lime trees with and without the application of synthetic elicitors.

	Leaf macronutrient concentrations				
	N (%)	P (%)	Ca (%)	K (%)	Mg (%)
Control	1.91 ± 0.06 ^a	0.15 ± 0.01 ^a	2.62 ± 0.27 a	1.80 ± 0.06 a	0.25 ± 0.01 a
Thiamine	2.09 ± 0.19 a	0.15 ± 0.01 a	2.72 ± 0.24 a	1.58 ± 0.04 b	0.28 ± 0.02 a
SA	2.00 ± 0.08 a	0.16 ± 0.01 a	2.89 ± 0.06 a	1.61 ± 0.05 ab	0.26 ± 0.01 a
Chitosan	1.99 ± 0.03 a	0.15 ± 0.01 a	2.42 ± 0.12 a	1.64 ± 0.02 ab	0.29 ± 0.02 a
Br	1.93 ± 0.04 a	0.15 ± 0.01 a	2.66 ± 0.13 a	1.67 ± 0.05 ab	0.29 ± 0.02 a
Treatments (T)	$F = 0.64$; df = 4,19; $P = 0.644$	$F = 0.76$; df = 4,19; $P = 0.571$	Statistical analysis $F = 0.68$; df = 4,19; $P = 0.621$	$F = 3.35$; df = 4,19; $P = 0.046$	$F = 1.56$; df = 4,19; $P = 0.249$
	Leaf micronutrient concentrations				
	Cu (mg·kg ⁻¹)	Fe (mg·kg ⁻¹)	Mn (mg·kg ⁻¹)	Zn (mg·kg ⁻¹)	B (mg·kg ⁻¹)
Control	6.34 ± 0.48 a	105.78 ± 15.37 a	25.27 ± 3.69 a	19.70 ± 0.95 a	64.27 ± 4.22 a
Thiamine	6.14 ± 0.48 a	108.90 ± 13.45 a	33.20 ± 2.38 a	20.62 ± 1.42 a	71.65 ± 4.39 a
SA	6.52 ± 0.32 a	97.07 ± 7.94 a	31.82 ± 4.15 a	18.02 ± 0.84 a	69.80 ± 2.52 a
Chitosan	6.92 ± 0.25 a	107.35 ± 6.26 a	29.12 ± 2.71 a	19.70 ± 0.49 a	72.20 ± 7.56 a
Br	6.83 ± 0.41 a	117.25 ± 12.30 a	33.77 ± 3.58 a	19.95 ± 0.58 a	78.17 ± 7.99 a
Treatments (T)	$F = 1.22$; df = 4,19; $P = 0.354$	$F = 0.30$; df = 4,19; $P = 0.873$	Statistical analysis $F = 2.14$; df = 4,19; $P = 0.138$	$F = 0.94$; df = 4,19; $P = 0.474$	$F = 1.43$; df = 4,19; $P = 0.284$

^aEach datum represents the mean of four values ±SE.

^bDifferent letters are significantly different, according to Tukey's test at $P \leq 0.05$.

Br = brassinosteroids; SA = salicylic acid.

from each tree. In all cases, leaves and terminal flush shoots were collected from the middle portion of the season's terminal growth. Leaves and flush shoots samples from the same treatment of each block were combined and collected in paper bags to perform a complete plant tissue analysis (nitrogen, phosphorus, potassium, calcium, magnesium, copper, iron, manganese, boron, and zinc), respectively. The samples were sent for analysis to the laboratory of the Faculty of Agricultural Sciences of the National University of Colombia in Bogotá.

Statistical design. A multi-observational *F*-protected analysis (a series of experiments) was used. For this reason, the experiments were arranged in a randomized block design in the different farms. In addition, untreated trees serving as the control were included in each block. The experimental unit in each farm was composed of three trees surrounded by guard trees and each treatment was repeated four times (four blocks). To analyze populations of *D. citri*, data were transformed ($\log X + 1$) because they were not normally distributed. Likewise, the percentage data were transformed using the arcsine formula. When differences were observed ($P \leq 0.05$) in an analysis of variance, the Tukey post hoc test was used to compare means. The data were analyzed using the statistical program Statistix (Version 9; Tallahassee, FL).

Results

Population dynamics of ACP individuals. The analysis of the population dynamics expressed as individuals accumulated throughout the test also showed differences in adults ($F = 2.89$; df = 4,175; $P = 0.024$) and nymphs ($F = 3.12$; df = 4,175; $P = 0.017$) accumulated per flush shoot from 2 WAT until the end of the experiment. On the other hand, the variable "accumulated eggs" was different from 1 WAT ($F = 6.17$; df = 4,175; $P = 0.000$) to the last week of the experiment (Table 2). In general, foliar applications of elicitors caused a lower accumulation of adults compared with the control. However, it was observed that the use of chitosan showed a greater tendency to reduce the number of adults accumulated (0.5 individuals) at the end of the trial in comparison with untreated trees (2.2 individuals) (Fig. 2A). Similar results were also registered on nymphs where SA (4.8 individuals) and thiamin (4.5 individuals) treatments had the lowest number of individuals at 4 WAT (10.2 individuals in untreated trees) (Fig. 2B). Also, all synthetic elicitors reduced the oviposition of *D. citri* by 30% (9.8 individuals in treated trees vs. 17 individuals in control trees) (Fig. 2C). Finally, ACP cumulative populations were higher in 'Rochela' farm than in 'Parcela 3' and 'Rochelita', with 20% adults (1.37 vs. 1.11 cumulative adults per flush), 35% nymphs (9.35 vs. 6.07 cumulative nymphs per flush), and 27% eggs (15.57 vs. 11.47 cumulative eggs per flush) at 4 WAT, respectively (Data not shown).

Physiological variables. No significant differences were found on g_s , leaf temperature,

Table 5. Concentration of macro- and micronutrients in flush shoots of Tahiti lime trees with and without applications of synthetic elicitors.

Flush macronutrient concentrations						
	N (%)	P (%)	Ca (%)	K (%)	Mg (%)	
Control	1.92 ± 0.12 ^b	0.21 ± 0.01 a ^y	1.62 ± 0.11 a	1.86 ± 0.08 a	0.19 ± 0.01 b	
Thiamine	2.45 ± 0.31 ab	0.27 ± 0.05 a	1.75 ± 0.15 a	1.72 ± 0.06 a	0.22 ± 0.01 ab	
SA	2.65 ± 0.23 a	0.26 ± 0.04 a	1.43 ± 0.20 a	1.86 ± 0.20 a	0.20 ± 0.01 ab	
Chitosan	2.33 ± 0.21 ab	0.25 ± 0.02 a	1.56 ± 0.20 a	1.85 ± 0.06 a	0.24 ± 0.01 a	
Br	2.23 ± 0.23 ab	0.22 ± 0.01 a	1.65 ± 0.10 a	1.79 ± 0.12 a	0.22 ± 0.01 ab	
Treatments (T)	$F = 4.33$; df = 4,19; $P = 0.021$	$F = 0.73$; df = 4,19; $P = 0.586$	Statistical analysis $F = 0.56$; df = 4,19; $P = 0.694$	$F = 0.26$; df = 4,19; $P = 0.894$	$F = 4.44$; df = 4,19; $P = 0.019$	
Flush micronutrient concentrations						
	Cu (mg·kg ⁻¹)	Fe (mg·kg ⁻¹)	Mn (mg·kg ⁻¹)	Zn (mg·kg ⁻¹)	B (mg·kg ⁻¹)	
Control	6.94 ± 0.37 a	47.65 ± 2.66 a	14.92 ± 0.78 a	23.10 ± 3.36 a	36.97 ± 4.22 a	
Thiamine	8.39 ± 0.36 a	59.62 ± 4.26 a	21.75 ± 3.08 a	28.70 ± 6.14 a	47.50 ± 6.88 a	
SA	7.99 ± 0.67 a	85.80 ± 18.12 a	16.10 ± 1.99 a	23.62 ± 3.15 a	38.55 ± 3.34 a	
Chitosan	8.12 ± 0.60 a	58.97 ± 6.70 a	17.05 ± 1.78 a	25.65 ± 4.50 a	44.15 ± 8.28 a	
Br	8.73 ± 0.07 a	63.17 ± 5.88 a	20.22 ± 3.73 a	18.95 ± 1.67 a	39.55 ± 3.05 a	
Treatments (T)	$F = 2.54$; df = 4,19; $P = 0.095$	$F = 2.58$; df = 4,19; $P = 0.090$	Statistical analysis $F = 2.31$; df = 4,19; $P = 0.117$	$F = 0.70$; df = 4,19; $P = 0.608$	$F = 0.57$; df = 4,19; $P = 0.687$	

^yEach datum represents the mean of four values ±SE.

Br = brassinosteroids; SA = salicylic acid.

chlorophyll content, and PSII efficiency among trees treated or not treated with foliar application of elicitors. In general, foliar applications had no effect on physiological variables in Tahiti lime trees (Table 3). However, significant differences were obtained in the RGR ($P = 0.044$) (Fig. 3), where foliar applications of Br promoted faster growth of flush shoots (44 mm·cm⁻¹·d⁻¹) compared with the control treatment and foliar application of chitosan (24 and 26 mm·cm⁻¹·d⁻¹, respectively).

The MDA content in both leaves and flush shoots of Tahiti lime trees was unaffected by elicitor treatments in the third experiment. However, a slight increase in lipid peroxidation was observed in leaves (4.5 μmol·g⁻¹ FW in control vs. 2.7 μmol·g⁻¹ FW in treated trees) and flush shoots of control trees (9.8 μmol·g⁻¹ FW in control vs. 7.5 μmol·g⁻¹ FW in sprayed trees). Nevertheless, proline synthesis was higher for the chitosan foliar applications where treated shoots had a higher content (41.39 and 34.53 μmol·g⁻¹ FW in flush shoots and leaves) (Fig. 4).

Foliar concentration of macro- and micro-nutrients was unaffected by the different treatments, except for potassium (K) (Table 4). Leaves of control trees had a higher leaf concentration of K (1.8%) compared with leaves treated with thiamin (1.58%), SA (1.61%), chitosan (1.64%), and Br (1.67%). Regarding the concentration of macro- and micronutrients in flush shoots, applications of the elicitors mainly increased the nitrogen and magnesium content. Control flush shoots showed a concentration of 1.92% nitrogen and 0.19% magnesium. Meanwhile, the average concentration of flush shoots treated with elicitors was 2.4% nitrogen and 0.22% magnesium (Table 5).

Discussion

Vitamins have been reported as resistance inducers under conditions of biotic stress (Boubakri et al., 2016). In this context, studies on the use of vitamins such as thiamine have mainly focused on the management of pathogens (Boubakri et al., 2012, 2016). However, recent research has shown that thiamine treatments in barley crops reduce aphid population by 60% compared with untreated plants (Hamada and Jonsson, 2013). Hamada et al. (2017) also found that thiamine treatments decrease the performance of generalist and specialist aphids in pea, barley, and wheat. Similar observations were made in the present study where foliar applications of thiamine caused a 50% reduction in ACP populations. Thiamine acts by signaling SA that can subsequently induce the transcription of the NPR1 gene, which causes host responses such as the production of phytoalexins, callose, and PR proteins (Boubakri et al., 2016). In this regard, PR proteins are known elicitors that can induce resistance through a variety of mechanisms such as cell wall reinforcement, glucanase and chitinase expressions, and nutrient remobilization (Sudisha et al., 2012).

Chitosan has been considered as an alternative to synthetic insecticides by several authors in the last decade (Badawy and El-Aswad, 2012; El Hadrami et al., 2010). Sharp (2013) mentions that the bioefficacy of chitosan has been demonstrated in insect pests of the orders Hemiptera and Lepidoptera. For example, Zhang et al. (2003) reported that applications of chitosan resulted in 60% and 80% mortality of six types of aphids (*Rhopalosiphum padi*, *Sitobion avenae*, *Metopolophium dirhodum*, *Myzus persicae*, *Hyalopterus pruni*, and *Aphis gossypii*) under laboratory conditions. However, field studies concerning the effectiveness of chitosan are limited and are mainly related to the management of *D. citri*. In this regard, foliar applications of chitosan caused a reduction of 40% to 70% of accumulated individuals (adults, nymphs, and eggs) of ACP (Fig. 2). Cooper and Horton (2017) observed that chitosan reduced populations of pear tree psyllid nymphs (*Cacopsylla pyricola*) by 30% in field trials. In addition, Cooper and Horton (2015) observed that pear trees treated with elicitors such as chitosan had a lower percentage of oviposition of *C. pyricola* females. Chitosan has been reported to bind to cell membranes causing the production of H₂O₂ through the octadecanoid pathway, favoring the biosynthesis of jasmonates that induce the expression of defense response genes (PR genes) (Pichyangkura and Chadchawan, 2015).

Phytohormones such as SA and Br also are important in signaling to activate resistance to arthropods (Erb et al., 2012). In the present study, SA and Br showed a positive effect in the control of *D. citri* individuals. Canales et al. (2016) mention that BR can favor the expression of genes associated with systemic acquired resistance. Likewise, Br can stimulate the synthesis of PR proteins by increasing SA (Bartwal et al., 2013). However, Shi et al. (2016) found that exogenous applications of SA caused reductions (70%) in *Bemisia tabaci* (Gennadius) individuals (an insect with a similar feeding habit as *D. citri*'s) on tomato plants. Among the defense mechanisms generated by SA, Smith et al. (2009) mentioned that this hormone could induce cell death of the affected area, limiting the feeding of the biotic agent. Also, SA is involved in the production and release of volatiles such as terpenes (Wu and Baldwin, 2009). Terpenes are important in the mediation of the plant–herbivory interaction (Gershenson and Dudareva, 2007). Shi et al. (2016) observed that exogenous applications of SA favored the release of volatile terpenes such as δ -limonene and methyl salicylate in tomato plants that caused repellence effect on *B. tabaci*.

Foliar chitosan sprays caused an increase in proline synthesis in both flush shoots and leaves of Tahiti lime. Studies carried out by Bistgani et al. (2017) also observed that applications of chitosan favored proline accumulation in *Thymus daenensis* Celak plants. Proline is an amino acid that promotes plant tolerance against stressful conditions because it acts as a signaling molecule that modulates mitochondrial functions, influences cell

proliferation or cell death, and triggers specific gene expression (Szabados and Savoure, 2010). Reports show that chitosan can alter the oxidative balance in the cytoplasm as a signaling mechanism to activate plant defense responses and reduce the symptoms and damages associated with biotic stress (Aranega-Bou et al., 2014). Herein, an explanation as to the increase in proline in flush shoots and leaves of Tahiti lime caused by the applications of chitosan may be because of its osmoprotectant function and may also signal hypersensitive responses against biotic stresses (Qamar et al., 2015).

The use of elicitors favored the growth of flush shoots in the present study. Some authors have stated that BR and SA actively participate in vegetative growth in plants (Clouse and Sasse, 1998; Rivas-San Vicente and Plasencia, 2011). Likewise, plant elicitors such as Br and SA can stimulate nitrate reductase activity under stressful conditions (Hayat et al., 2012, 2014). In addition, elicitors can also promote the mobilization of N for the production of defensive metabolites such as alkaloids and polyamines against biotic stressors (Mur et al., 2017). This can explain to certain extent why flush shoots treated with plant elicitors showed a higher growth and N concentration in the present study.

In summary, the development of IPM programs is of vital importance to the management of the ACP. Thus, the use of synthetic elicitors can be considered as a tool to minimize the applications of chemical insecticides and decrease the development of resistance in *D. citri* because the use of these compounds reduced ACP populations between 30% and 70% in all development stages (adults, nymphs, and eggs). Although plants reported level of stress recorded by the increase of secondary metabolites synthesis (proline), the use of these products have positive effects on plant physiology because they favored flush vegetative growth and nitrogen concentration. More studies are needed to determine the duration of efficacy of synthetic elicitors, quantify other physiological effects such as yield and quality of the fruit, and study the interaction between ACP and its natural enemies.

Literature Cited

- Agronet. 2017. Red de información y comunicación del sector agropecuario. 12 Mar. 2018. <<http://www.agronet.gov.co/Paginas/default.aspx>>.
- Ahn, I.P., K. Soonok, and Y.H. Lee. 2005. Vitamin B1 functions as an activator of plant disease resistance. *Plant Physiol.* 138:1505–1515.
- Angelova, Z., S. Georgiev, and W. Roos. 2006. Elicitation of plants. *Biotechnol. Equip.* 20:72–83.
- Aranega-Bou, P., M. de la O Leyva, I. Finiti, P. García-Agustín, and C. González-Bosch. 2014. Priming of plant resistance by natural compounds. Hexanoic acid as a model. *Front. Plant Sci.* 7:5.
- Badawy, M.E.I. and A. El-Aswad. 2012. Insecticidal activity of chitosans of different molecular weights and chitosan-metal complexes against cotton leafworm *Spodoptera littoralis* and oleander aphid *Aphis nerii*. *Plant Protection Sci.* 48:131–141.
- Bartwal, A., R. Mall, P. Lohani, S.K. Guru, and S. Arora. 2013. Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. *J. Plant Growth Regulat.* 32:216–232.
- Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207.
- Bistgani, Z.E., S.A. Siadat, A. Bakhshandeh, A.G. Pirbalouti, and M. Hashemi. 2017. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. *Crop J.* 5:407–415.
- Boubakri, H., M. Gargouri, A. Mliki, F. Brini, J. Chong, and M. Jbara. 2016. Vitamins for enhancing plant resistance. *Planta* 244:529–543.
- Boubakri, H., M.A. Wahab, J. Chong, C. Bertsch, A. Mliki, and I. Soustre-Gacougnolle. 2012. Thiamine induced resistance to *Plasmopara viticola* in grapevine and elicited host-defense responses, including HR-like-cell death. *Plant Physiol. Biochem.* 57:120–133.
- Campos, M.L., M. de Almeida, M.L. Rossi, A.P. Martinelli, C.G.L. Junior, A. Figueira, V.T. Rampelotti-Ferreira, J.D. Vendramin, F.A. Benedito, and L.E. Peres. 2009. Brassinosteroids interact negatively with jasmonates in the formation of anti-herbivory traits in tomato. *J. Expt. Bot.* 60:4347–4361.
- Canales, E., Y. Coll, I. Hernández, R. Portieles, M. Rodríguez García, Y. López, M. Aranguren, E. Alonso, R. Delgado, M. Luis, L. Batista, C. Paredes, M. Rodríguez, M. Pujol, M.E. Ochagavía, V. Falcón, R. Terauchi, H. Matsumura, C. Ayra-Pardo, R. Llauger, C. Pérez Mdel, M. Núñez, M.S. Borrusch, J.D. Walton, Y. Silva, E. Pimentel, C. Borroto, and O. Borrás-Hidalgo. 2016. 'Candidatus liberibacter asiaticus', causal agent of citrus huanglongbing, is reduced by treatment with brassinosteroids. *PLoS One* 11: e0146223.
- Clouse, S.D. and J.M. Sasse. 1998. Brassinosteroids: Essential regulators of plant growth and development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49:427–451.
- Cooper, W.R. and D.R. Horton. 2015. Effects of elicitors of host plant defenses on pear psylla, *Cacopsylla pyricola*. *Entomol. Expt. Appl.* 157(3):300–306.
- Cooper, W.R. and D.R. Horton. 2017. Elicitors of host plant defenses partially suppress *Cacopsylla pyricola* (Hemiptera: Psyllidae) populations under field conditions. *J. Insect Sci.* 17:49.
- El Hadrami, A., L.R. Adam, I. El Hadrami, and F. Daayf. 2010. Chitosan in plant protection. *Mar. Drugs* 8:968–987.
- Erb, M., S. Meldau, and G.A. Howe. 2012. Role of phytohormones in insect-specific plant reactions. *Trends Plant Sci.* 17:250–259.
- García-Castro, A., A. Volder, H. Restrepo-Díaz, T.W. Starman, and L. Lombardini. 2017. Evaluation of different drought stress regimens on growth, leaf gas exchange properties, and carboxylation activity in purple passionflower plants. *J. Amer. Soc. Hort. Sci.* 142:57–64.
- Gershenson, J. and N. Dudareva. 2007. The function of terpene natural products in the natural world. *Nat. Chem. Biol.* 3(7):408–414.
- Goyer, A. 2010. Thiamine in plants: Aspects of its metabolism and functions. *Phytochemistry* 71:1615–1624.
- Halbert, S.E., X. Sun, and W.N. Dixon. 2000. Asian citrus psyllid and citrus greening disease. *Citrus Ind.* 91:22–24.

- Hamada, A.M., J. Fatehi, and L.M.V. Jonsson. 2017. Seed treatments with thiamine reduce the performance of generalist and specialist aphids on crop plants. *Bul. Entomol. Res.* 108:1–9.
- Hamada, A.M. and L.M.V. Jonsson. 2013. Thiamine treatments alleviate aphid infestations in barley and pea. *Phytochemistry* 94:135–141.
- Hayat, S., M.N. Alyemeni, and S.A. Hasan. 2012. Foliar spray of brassinosteroid enhances yield and quality of *Solanum lycopersicum* under cadmium stress. *Saudi J. Biol. Sci.* 19(3):325–335.
- Hayat, S., Q. Hayat, M.N. Alyemeni, and A. Ahmad. 2014. Salicylic acid enhances the efficiency of nitrogen fixation and assimilation in *Cicer arietinum* plants grown under cadmium stress. *J. Plant Interact.* 9(1):35–42.
- Hodges, D.M., J.M. DeLong, C.F. Forney, and R.K. Prange. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 207:604–611.
- ICA. 2015. Resolución 2390 de 2015: “Por medio de la cual se declara el estado de emergencia fitosanitaria en el territorio nacional por la presencia de adultos de *Diaphorina citri* infectados con la bacteria de la enfermedad del HLB de los cítricos”. 12 Mar. 2018. <<http://www.ica.gov.co/Normatividad/>>.
- Li, J., P. Trivedi, and N. Wang. 2016. Field evaluation of plant defense inducers for the control of citrus huanglongbing. *Phytopathology* 106:37–46.
- Mitchell, C., R.M. Brennan, J. Graham, and A.J. Karley. 2016. Plant defense against herbivorous pests: Exploiting resistance and tolerance traits for sustainable crop protection. *Front. Plant Sci.* 7:1132.
- Mur, L.A., C. Simpson, A. Kumari, A.K. Gupta, and K.J. Gupta. 2017. Moving nitrogen to the centre of plant defence against pathogens. *Ann. Bot.* 119:703–709.
- Pawlowski, M.L., C.R. Bowen, C.B. Hill, and G.L. Hartman. 2016. Responses of soybean genotypes to pathogen infection after the application of elicitors. *Crop Protection* 87:78–84.
- Peng, J., X. Deng, J. Huang, S. Jia, S. Miao, and Y. Huang. 2004. Role of salicylic acid in tomato defense against cotton bollworm, *Helicoverpa armigera* Hubner. *Z. Naturforsch. C* 259:856–862.
- Pichyangkura, R. and S. Chadchawan. 2015. Bio-stimulant activity of chitosan in horticulture. *Scientia Hort.* 196:49–65.
- Pluke, R.W.H., J.A. Qureshi, and P.A. Stansly. 2008. Citrus flushing patterns, *Diaphorina citri* (Hemiptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae) in Puerto Rico. *Florida Entomol.* 91:36–42.
- Qamar, A., K.S. Mysore, and M. Senthil-Kumar. 2015. Role of proline and pyrroline-5-carboxylate metabolism in plant defense against invading pathogens. *Front. Plant Sci.* 6:503.
- Rehman, F., F.A. Khan, S.B. Anis, and A.A. Ansari. 2016. Plant defense response against grasshopper herbivory. *J. Entomol. Zool. Stud.* 4:184–190.
- Richter, K. and J. Koolman. 1991. Antiecdysteroid effects of brassinosteroids in insects, p. 265–278. In: H.G. Cutler, T. Yokota, and G. Adam (eds.). *Brassinosteroids. Chemistry, bioactivity, and applications*. American Chem. Soc., Washington, DC.
- Rivas-San Vicente, M. and J. Plasencia. 2011. Salicylic acid beyond defence: Its role in plant growth and development. *J. Expt. Bot.* 62:3321–3338.
- Santivañez, T., G. Mora Aguilera, G. Diaz Pinilla, J.I. Lopez Arrollo, and P. Vernal Hurtado. 2013. Citrus. Marco estratégico para la gestión regional del Huanglongbing en América Latina y el Caribe. FAO, Santiago de Chile, Chile.
- Sétamou, M., D. Flores, J.V. French, and D.G. Hall. 2008. Dispersion patterns and sampling plans for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus. *J. Econ. Entomol.* 101:1478–1487.
- Sharp, R.G. 2013. A review of the applications of chitin and its derivatives in agriculture to modify plant-microbial interactions and improve crop yields. *Agronomy (Basel)* 3: 757–793.
- Shi, X., G. Chen, L. Tian, Z. Peng, W. Xie, Q. Wu, and Y. Zhang. 2016. The salicylic acid-mediated release of plant volatiles affects the host choice of *Bemisia tabaci*. *Intl. J. Mol. Sci.* 17:1048.
- Smith, J.L., C.M. De Moraes, and M.C. Mescher. 2009. Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. *Pest Mgt. Sci.* 65:497–503.
- Sudisha, J., R.G. Sharathchandra, K.N. Amruthesh, A. Kumar, and H.S. Shetty. 2012. Pathogenesis related proteins in plant defense response, p. 379–403. In: J. Méillon and K. Ramawat (eds.). *Plant defence: Biological control. Progress in biological control*, vol 12. Springer, Dordrecht, The Netherlands.
- Szabados, L. and A. Savoure. 2010. Proline: A multifunctional amino acid. *Trends Plant Sci.* 15(2):89–97.
- Thakur, M. and B.S. Sohal. 2013. Role of elicitors in inducing resistance in plants against pathogen infection: A review. *ISRN Biochem.* 2013:1–9.
- Tiwari, S., W.L. Meyer, and L.L. Stelinski. 2013. Induced resistance against the Asian citrus psyllid, *Diaphorina citri*, by β -aminobutyric acid in citrus. *Bul. Entomol. Res.* 103:592–600.
- Tsagkarakis, A.E., M.E. Rogers, and T.M. Spann. 2012. Applications of plant growth regulators to container-grown citrus trees affect the biology and behavior of the Asian Citrus Psyllid. *J. Amer. Soc. Hort. Sci.* 137:3–10.
- Wei, H., J. Zhikuan, and H. Qingfang. 2007. Effects of herbivore stress by *Aphis medicaginis* Koch on the malondialdehyde contents and the activities of protective enzymes in different alfalfa varieties. *Acta Ecol. Sin.* 27:2177–2183.
- Wu, J.Q. and I.T. Baldwin. 2009. Herbivory-induced signalling in plants: Perception and action. *Plant Cell Environ.* 32:1161–1174.
- Zhang, M.I., T. Tan, H. Yuan, and C. Rui. 2003. Insecticidal and fungicidal activities of chitosan and oligo-chitosan. *J. Bioact. Compat. Poly.* 18:391–400.